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# Gene Editing Using CRISPR/Cas9 System in the Treatment of HIV and Cancers

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**Abstract:**

CRISPR/Cas9 has proved its revolutionary power in treating difficult diseases (such as HIV and cancer) with fresh horizons for precise medicine. CRISPR/Cas9 as a powerful tool are currently under investigation in the fields of HIV cure, for its ability to knock-out viral genome by editing host cell genes through disrupting the viral reservoir. In the cancer therapy, its aims are to boost the anti-tumor action of the immune cells (e.g. CAR-T cells) or direct the cancer gene mutation site to make the cancer cell apoptosis, but its off-target effect, in vivo delivery efficiency and the problem of longterm safety also are needed to solve. In this paper, we systematically review CRISPR/Cas9 research in HIV antiviral immune activation, the engineering on cancer immune cells, and tumor genome editing. It revises its performance in in vitro cells and animals and the technical challenges it confronts and points out the targeting capability and editing efficiency of the delivery system as two key factors determining its clinical translation. The study gives a theoretical guide for optimization of gene editing strategy which might be helpful for the development of safe and efficient gene therapy. This also underscores the necessity for future research to focus upon the design of smart delivery vectors, the generation of multi-editing combinatorial therapies and the long-term toxicity studies – all of which urgently need further interdisciplinary efforts to enable this technology to be translated from the lab, to the clinic.

**Keywords:**-CRISPR/Cas9; HIV; cancer; gene editing; cancer immune cells engineering

## I. Introduction

HIV infection and cancer are two public health risks threatening human's health in global area. Conventional treat-ment methods, e.g., ART, have the risk of drug resistance to HIV therapy, and surgery, radiotherapy, and chemothera-therapy have limited efficiency with heavy side effects for cancer treatment. Thus, the development of more effective and accurate new treatment strategies is urgently required.

With the development of gene editing technology, it has opened revolutionary prospects for the HIV and cancer. From the preliminary zinc finger nucleases (ZFNs) and transcription activatorlike effector nucleases (TALENs), to the lately extraordinarily acclaimed CRISPR/Cas9 and related protein, the technology of gene editing has been constantly evolving. Among them, CRISPR/Cas9 is with its new mechanism obtained from bacteria immune system to form a complex with the guide RNA (gRNA) and Cas9 protein to precisely locate and cut target DNA sequences for gene knockout, insertion or replacement. CRISPR/Cas9 has the advantages over ZFNs and TALENs that simple design, low cost, high efficiency, target multiple gene sites at the same time, therefore, it is a hot research area in the field of life science with fast progress.

Now, CRISPR/Cas9 technology also breaks through in treating of HIV or cancer. In treating HIV, the scientists knock out host cells' CCR5 gene, because CCR5 is the main entry point for the HIV virus, or directly cut target HIV or viral DNA that integrated to the human genes aim at achieving function cure. In the context of treatment for cancer, CRISPR/Cas9 is employed in editing immune cell genes in order to further these cells' capacity to identify tumor cells and kill them, or knock out the oncogenes in tumor cells so as to impede tumor proliferation and metastasis. In fact, we still have far to go before CRISPR/Cas9 can be fully applied to cancer treatment clinically. This mainly comes from the possible mutations caused by gene off-target effects, troubles related to the safety and efficiency of delivery systems and the social pressure arising from ethical controversies.

According to the hot research status, this paper will take specific CRISPR/Cas9 applications in the treatment of HIV and cancer as an example to analyse from aspects of technologies, summarise the technical advantages and existing problems, combine new research achievements and put forward the ways of optimization and development trends. The present study can not only be helpful for further exploring the great possibility of CRISPR/Cas9 to cure the major diseases, but also give theoretical guidance for breaking through the technical hurdles and accelerating gene editing therapy into clinical applications. It has far practical significance for HIV patient and cancer patient to increase their living standards of life and solving world health problems.

## II. Introduction to CRISPR/Cas9

### A. Composition and Principle of CRISPR/Cas9

CRISPR/Cas9, derived from the pathogenic immune system of viruses, works based on the principle that the guide RNA(gRNA) assisted Cas9nuclease can recognize and cut the target DNA by artificial design. The guide RNA includes complementary sequences to the target DNA, and only when the target DNA is bound, the gRNA can recognize the downstream PAM sequence( e.g. NGG) and makes the target DNA cut precisely. Once Cas9 cleaves the DNA, generating double-strand breaks, the cell repairs the DNA with the nonhomologous endjoining (NHEJ) repair system. This technique is able to take advantage of the natural defence system against viruses and precisely editing the genes by designing specific gRNA [1].

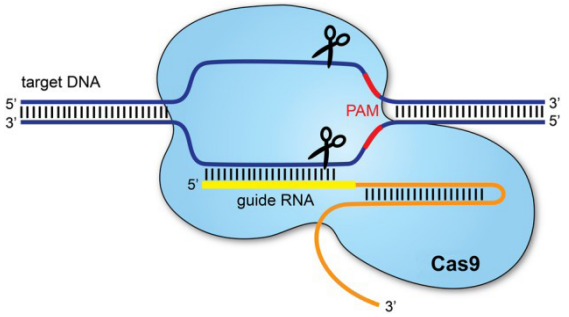


**Figure1. CRISPR/Cas9 System [1].**

As shown in Figure 1, in the CRISPR/Cas9 system, CRISPR refers to the clustered regularly interspaced short palindromic repeats (CRISPR-RIs) found in the bacterial genome. When bound to a series of CRISPR-related proteins, such as Cas9, CRISPR can defend against viral invasions. Cas9 is a nuclease that can cut double-stranded DNA. It is directed to the target by a synthetic single-guide RNA (sgRNA), where the RNA segment that binds to the genomic DNA is 18-20 nucleotides long. For cleavage to occur, there must be 2-5 nucleotides of specific DNA sequence (PAM) adjacent to the 3' end of the guide RNA. After DNA cleavage, two repair pathways are available: non-homologous end joining typically results in random insertions or deletions of DNA, while homologous directed repair uses a homologous DNA segment as a template for repair. This method can achieve precise genome editing, similar to the precision of single base pair edits, by delivering Cas9 nuclease, sgRNA, and DNA homologous segments with the required sequence changes [2].

## III. Application of CRISPR/Cas9 in HIV treatment

The existing mechanism and mode of action: CRISPR/Cas system is bacterial adaptive immune mechanism which protects bacteria from exogenous nucleic acid attacks by having 3 phases, the adaptation, the expression, the interference. The most popular are the so-called II-

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class (Cas9/gRNA), which utilize nuclease activity of the RuvC and HNH domains. Mutations in these domains (D10A/H840A) yields dCas9 which still binds to DNA but not capable of cleaving. For HIV therapy, this system has been worked on three ways: Targeting HIV genome: some gRNAs are designed to guide Cas9 to cleave and insert into the HIV proviral DNA of the host genome, for example, target the long terminal repeat (LTR) of HIV-1 in order to disrupt viral transcription initiation and inhibit replication [3]. Essential auxiliary receptor genes for HIV infection (e.g., CCR5) are knocked down or mutated to render the host cells resistant from R5-type virus infection; dCas9 fused with transcription activation domains (e.g., SAM system) binds on the LTR region of the latent viruses, and activates the viral transcription and removes the latent virus reservoir by 'shaking, killing' strategy [4].As traditional antiretroviral therapy (HAART) cannot eliminate the viral reservoir in latent form, but CRISPR/Cas9 could cleave pre-viral DNA from a genetic angle, maybe it can be a functional cure. It has been proved that it can target viral genomes or host factor and inhibit the infection and degrade the latent virus [5]. In order to cope with the high genetic diversity of HIV, it is possible to use more than one gRNAs targeting conserved viral regions, or combining HIV-1 therapy with HIV host factor editing to decrease risks of viral mutation and escape. For instance, targeting more key viral genes will increase treatment robustness; Delivery safety and efficacy are the critical factors for clinical translation. Viral vectors (e.g. AAV and lentiviruses) suffer from the limitation in packing capacity and immunogenicity and non-viral vectors (e.g. lipid nanoparticles) still need to address low delivery efficiency. Delievry system optimization is the current research focus [6]. Viral escape, immunogenicity and long-term safety are key issues. Off-target effect refers to editing of non-target gene sequences and may cause severe off-target effects in host genome due to unintended off-target cuts. The origin of bacterial Cas9 would induce immune response.

Ebina et al. used SpCas9/gRNA to target the NF-κB site in the LTR of HIV-1, reducing viral gene expression by 45.6% to 90% in Jurkat cells, confirming for the first time that Cas9 can cut pre-viral DNA. Hu used dual gRNAs to target the LTR conservation region, removing 9709 bp of viral sequence from latent infection T cells and monocytes, completely inhibiting replication without off-target effects [7]. Xu et al. knocked out the CCR5 gene in human CD34+ hematopoietic stem cells and transplanted them into mice, successfully transforming the cells to resist R5-type HIV infection and maintain long-term hematopoietic function [8].

Yin et al. used AAV to deliver SaCas9 and quadruple gRNA to Tg26 mice carrying HIV pre-virus, resulting in a 80%-90% reduction in HIV DNA in tissues such as the spleen and liver, and a decrease in viral RNA expression. This was the first demonstration of the feasibility of in vivo pre-virus clearance [9]. Dash et al. combined long-term ART with CRISPR treatment in humanized mice, and the viral load in the dual therapy group was significantly lower than in the single therapy group, with some mice showing undetectable viral loads [10].

Zhang et al. have used the dCas9-SAM system to awaken latent Jurkat cells, there by amplifying 10-20 folds of viral RNA and protein expression, was supported for the 'shaking, killing' approach [11]. The target specificity and multiplexed function, targeting viral conserved sequence to minimize off-target risk, destroying viral DNA, editing host factor, or awaking the viral latency, which tackles with the complicated HIV life cycle. Combined with ART and immunotherapies (e.g. CAR-T) which can achieve synergistic effects to inhibit replication and clear the latently infected virus reservoir. Therefore, optimal designing of editing tools, design of novel variants of Cas12a and Cas13 as well as base editing and Prime Editing technologies to enhance efficiency and diminish off-target effects. Development of targeting delivery vectors such as exosome for enhancing specificity and diminishing immunogenicity. Conduct further long-term safety assessment in non-human primate model and assess the possible enhanced functional "edit, clear" "edit, clear" combinatorial approach (ex., CRISPR + CAA-T) in vivo. CRISPR/Cas9 has received considerable attention as a platform to enhance the current HIV treatment paradigm by potentially eliminating the pre-existing reservoir of the virus and to treat the latent infected cells in vivo, with clear evidence of therapeutic efficacy, however, this technology is not without issues associated with off-target effects, delivery, and immune responses. Optimization of tools, innovations in delivery mechanisms, and development of new interdisciplinary relationships all hold promise to accelerate clinical translation and establish the basis for eradicating HIV/AIDS.

## IV. Application of CRISPR/Cas9 in cancer therapy

### A. Application In Cancer Drug Development

Genes or proteins to be targeted must be accurately identified for cancer drug development. CRISPR Cas9 could be directly used for genome editing, thus researchers could find defective genes and analyze their behaviours, so as to systematically identify genes that sustain cancer cell

survival and modulate drug sensitivity. The technology is developed on the adaptive immune system of bacteria with gRNA guiding Cas9nuclease to target DNA sequence for the execution of exact gene knockout, insertion, modification, etc. The CRISPR Cas9 technology has laid out new directions on the functional genomics research and has provided some potential in the cancer drug development. It surmounts drawbacks of conventional screening approaches, such as RNAi-based gene knockdown libraries and cDNA-based gene overexpression libraries, more promising to hit promising drug targets. LOF (Loss of Function) CRISPR and GOF (Gain of Function) CRISPR libraries were established in both experiments. Research conducted positive and negative selection tests based on the LOF CRISPR library, while we built the GOF CRISPR library and applied it, which has its superiority to cDNA traditional ones. Based on CRISPR knockout and CRISPR interference (CRISPRi), we screened out the large-scale sgRNA libraries for important cancer cells survival genes. The results have correctly detected the majority of genes linked to cancer (such as the genes BCR and ABL from chronic myeloid leukemia KBM7 cell line, as they result in cancer mortality (lethality) and those KRAS and PIK3CA for the DLD 1 and the cell HCT116 colorectal cancer with important functions in DNA replication, in the RNA process and protein destruction. But the CRISPR system has also been found to exhibit offtarget effect, which is an important hindrance to the application of the CRISPR system. CRISPR Cas9 technology is powerful in searching targets for cancer drug development, allowing the precise editing of the genomes and identifying cancer-related important genes, providing a firm foothold for subsequent drug development. However, no off-target effects should be neglected; if not handled properly, they will cause inaccuracies and unreliability of those targets selected by this technology, which will also inhibit cancer drug development. Off-target effect must be minimized and its value enlarged in the future development of cancer drug with the technology optimization [12].

## B. Application of CRISPR/Cas system in Hepatocellular Carcinoma

The existing principle is that the CRISPR/Cas system employs Cas9 nuclease to cleave the DNA double strand with the instructions from sgRNAs. The damaged DNA will be repaired by nonhomologous end joining (NHEJ) or accurate homologous recombination (HR) later. NHEJ generally leads to loss of gene function while HR can perform site directed mutation. Research purpose: The aim of this research is to employ the CRISPR/Cas system to knockout cancersensitive genes in adult mouse liver, create a liver cancer model, and verify it is feasible, and offer new ways for cancer experiments. The experimental scheme constructs vectors, vector containing Cas9 and the targets Pten, p53 and Ctnnb1 (pX330), and Cas9D10Anickase vector. In vivo experiments: tail vein injection of plasmids into the livers of adult FVB mice, including groups for singlegene editing (Pten, p53), double-gene editing (Pten+p53) and the Ctnnb1 site-directed mutagenesis (with ssDNA binding), a control group. Methods to detect including immunohistochemistry (IHC), oil red staining. bioluminescence imaging, gene sequencing and off-target effect analysis. With regard to Pten editing, hepatocytes displayed negative Pten expression, Akt phosphorylation upsurges, the lipid accumulation phenomenon as well as notably high indel mutation rate, with most of them being missense mutations. With regard to p53 editing, single gene editing did not trigger tumor, while after 3 months' co-editing with Pten they all developed liver tumors with bile duct differentiation and double allele target gene. To investigate for Ctnnb1 editing, β-Catenin nuclear localization and the level of expression of the target gene GS increased, and the expected point mutations were induced successfully. The off-target frequency of sgPten is lower than the detection level, and it is safe enough. In this method, liver tumor is successfully induced without a need to use embryo stem cells manipulation, it is fast to test the functions for cancer genes. Delivery technology has to be further developed, side effects have to be analysed and a bigger range of targeted tissue has to be reached [13].

## C. Application in Breast Cancer

Now, the default rule is that CDH1 loss is the defining property of ILC (infiltrating lobular breast cancer) and the aetiology usually involves the CDH1gene mutation in concert with another mutation in e.g. PTEN. The present study achieves simulations of human ILC development by intraductal injection of lentiviruses (that include CRE recombinase or CRISPR/Cas9 systems) that selectively mutate genes within adult mouse mammary tissue. Note: Cas9 may induce immunological response, while conditional Cas9 expression (e.g., activating its expression in mammary cells) can produce immunological tolerance. For major editing, by injecting Cre virus into duct, the purpose of stimulating ILC can be realized. By injecting Cre virus into ILC in E-cadherin and PTEN/carcinogen-deficient mice, typical ILC pathological manifestations are induced, which has been proven by the conventional transgenic models. CRISPR needs to steer clear of the immune reaction caused by Cas9,and by directly injecting Cas9-carrying viruses can lead to high immune

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infiltration which induced non-ILC tumor, using mice already pre-expressing Cas9 (WapCre; Cdh1F/F; Cas9) and only injecting sgRNAvirus can achieve good induction of ILC with weak immune response. With this platform, rapid gene validation is enabled, and without any complicated breeding, the candidate tumor suppressor genes (like PTEN) can be assessed for whether they can work synergistically with the deficiency of E-cadherin in mice via viral injection.

To confirm the efficacy of Cre virus-mediated induction of ILC in Cdh1F/F; Akt E17K and Cdh1F/F; PtenF/F (in carrying conditional alleles) mice, we injected intraductally Lenti Cre virus.As previously reported, typical ILC following injection were present in the mammary glands of mice, in terms of single cell infiltrates, deposition of collagen, absence of E-cadherin, and induction of the PI3K/AKT pathway, identical to that reported using traditional WapCre transgenic models. The drawbacks of direct delivery of CRISPR/Cas9 are: direct intratumor injection of pSECC virus that encodes Cas9 and sgPTEN in to Cdh1F/F mice did lead to tumors $\sim25\%$ of the glands, but these were non-ILC phenotypes, with incomplete E-cadherin loss and extensive immune cell infiltration (CD4+, CD8+, B220+) indicating that Cas9 induces an immune response that disrupts normal tumor formation.In order to improve the conditional Cas9 model, we injected only the LentiGuide virus harboring sgPTEN (without Cas9) into the WapCre; Cdh1F/F; Cas9 mice that already preexpress Cas9. Thirty percent or so glands displayed characteristic ILCs, and there was minimal immune infiltration. PTEN was lost, and the PI3K/AKT pathway was activated, which suggested that the expression of conditional Cas9 is able to modulate the immune response. Conditional allele recombination: Efficient inducible transduction of epithelial cells in the breast is readily achieved with Lenti Cre injection into ducts15, enabling the creation of a sporadic model without the need for convoluted breeding schemes.

Cas9-exposed in exogenous immune response, it generates pathologic tumor microenvironment. However, Ecadherin- deleted in exogenous conditional expression of Cas9, exogenous Cas9 (like WapCre at early breast development), it is leading to generate immune tolerance and editing CRISPR by useful purpose. The missing of single gene E-cadherin can't trigger cancer (lead to cell apoptosis) but combine with tumor suppressor-gene, such as PTEN.PTEN loss as a major driver in IL- C Finally, CRISPR experiments have proven that PTEN loss is a major driver in ILC. They have designed a CRISPR/Cas9 somatic cell editing platform based on intraductal virus injection to rapidly generate ILC models in mice. The most important is resolution of Cas9's immunogenicity problems through conditional expression allowing injection of only sgRNA to efficiently induce ILC.Such a platform offers an effective instrument to comprehend the pathogenesis of ILC, to corroborate multi-gene synergy and to devise targeted therapies, which can be expanded to other breast cancer subtypes [14].

## D. Application in Bladder Cancer

The principle is to verify the role of the CRISPR/Cas9 system in regulating lncRNA UCA1 expression, explore the function of UCA1 in the development and progression of bladder cancer, and assess the clinical significance of urine UCA1 as a non-invasive diagnostic marker for bladder cancer. In vitro experiments involved designing 8 gRNAs targeting UCA1 and constructing a CRISPR/Cas9 system, which were then transfected into 5637 and T24 bladder cancer cell lines. Various methods, including T7 exonuclease 1 detection, DNA sequencing, quantitative RT-PCR, MTT assay, cell cycle analysis, apoptosis analysis, wound healing assay, Transwell assay, and gelatinase spectrum analysis, were used to detect changes in UCA1 expression, cell proliferation, migration, invasion, and related protein activity. In vivo experiments involved subcutaneously implanting 5637 cells transfected with CRISPR/Cas9 UCA1 (1+8) or gRNA empty vector into nude mice, regularly measuring tumor volume, and performing H&E staining, immunohistochemistry, and Western blot analysis on tumor tissue after the experiment [15]. The results of the experiment showed that UCA1 expression was inhibited. All 8 gRNAs reduced the UCA1 expression level in 5637 cells, with UCA1 1gRNA and UCA1 8 gRNA showing significant inhibitory effects. The co-transfection of CRISPR/Cas9 UCA1 1 and UCA1 8 can produce a synergistic inhibitory effect, which is due to the specific cleavage of the DNA encoded by UCA1. Malignant cell phenotype changes: Downregulation of UCA1 significantly inhibits the proliferation, migration, and invasiveness of 5637 and T24 bladder cancer cells, causing the cell cycle to be arrested in the G0/G1 phase, inducing apoptosis, and reducing MMP2/9 activity. When nude mice were inoculated with UCA1-deleted cells, tumor growth was slow, cell proliferation decreased, and the expression of MMP2, MMP9, and the anti-apoptotic protein Bcl-2 decreased, while the pro-apoptotic protein Bax increased. Six studies involving 619 bladder cancer patients and 491 healthy individuals were included. The analysis showed that urine UCA1 has a high diagnostic accuracy for bladder cancer, with a sensitivity of 0.83 [15].

CRISPR/Cas9 technology can effectively knock down the expression of lncRNA UCA1 which acts as an oncogene involved in bladder cancer cell proliferation, migration and invasion; Urinary UCA1 is suitable as a non-invasive

indicator for bladder cancer, it provide a new potential index of early diagnosis of bladder cancer and may help improve clinical management of bladder cancer.

## V. Limitations of CRISPR/Cas9

CRISPR/Cas9 induce appreciable levels of off-target effects, including important genes mutations and chromosomal translocations. Although results demonstrate that off-target cleavage is less common than with other nucleases, off-target effects cannot be disre garded, and several strategies have been exploited aiming to limit them, by using FokI dCas9 nucleases (RFNs) guided by dimer dependent RN and truncated guide RNAs (tru gRNAs). Nevertheless, a few strategies might induce cellular immune response or cytotoxicity. The Cas9/gRNA system effectively targets HIV-1, and the accompanied NHEJ repair pathway may cause the virus escape mutations. When targeting the non-essential viral sequences, the virus would escape immediately; and when targeting the conserved HIV-1 protein coding sequences, it can substantially slow virus escape, and the inhibitory rate is comparable to certain antiviral shRNAs targeting HIV-1 conserved regions. Lentiviruses, adeno-associated viruses, and adenoviruses are the major delivery vectors. The adenovirus recombinant vectors are hard to produce and the lentiviruses can integrate into host genome with the risk of off-target delivery. Adeno-associated viral vectors are limited by their small packaging size, immune response following repeated usage. Furthermore, the delivery efficiency of non-viral vectors like cationic polymers, lipid reagents and nanoparticles should be enhanced and there exists a blood-brain barrier blockage when delivering to the brain. CRISPR/Cas9 technology has a wide prospect in gene therapy, but there are also a lot of ethical controversies associated with this technology. The issue of CRISPR/Cas9 on human cells raised a lot of ethical controversy when the research was expanding to applying human cells. In 2015, Doudna conducted a conference and published a report against editing genes in human embryo cells. As for legal laws about editing human embryo cells, the laws in different countries have a different background, and heritable gene editing of embryos is complex. Although genetic editing of human somatic cells is less controversial compared with human embryos and tends to be more popular now, its high-cost feature also hinders its popularization. How this kind of technique will deal with ethical dilemmas, is an urgent topic in which both science and society must study and legislate together [16].

## VI. Conclusion

This article reviews the application of CRISPR/Cas9 in HIV and cancer therapeutic using CRISPR/Cas9. It introduces the technology principles (using guide RNA to cut the target DNA precisely with Cas9 nuclease), targeting the viral genome for HIV therapeutic; targeting host cell factors (e.g. knocking out the CCR5 gene) to eliminate the viral reservoir and inducing the latent virus reservoir. It is used in the treatment of cancer, for the development of drugs (screening key target gene, e.g. BCR, ABL, KRAS) and building models for liver, breast and bladder cancer (e.g. editing gene, e.g. Pten, CDH1, UCA1).The article describes the validation of effectivity of in vitro cell experiments and animal models (e.g. Tg26 mice, humanized mice).Our major results show that CRISPR/Cas9 can considerably suppress viral replication and clear latent virus reservoirs in the case of HIV treatment, and the joint use with long lasting ART therapy is further efficacious. In cancer treatment it can precisely detect oncogenes, induce tumor phenotypes and confirm the synergistic action of multiple genes (i.e., synergistic carcinogenesis of E-cadherin and PTEN loss in ILC), and urine UCA1 can function as a non-invasive diagnostic marker for bladder cancer. The benefits for this technology comprise low threshold of design and high editing efficiency and multifunctionality, etc., and its drawbacks in turn comprise the off-target effect (such as inducing genomic mutation), the immunogenicity of viral vector, a low delivery efficiency of non-viral vector (such as the blood-brain barrier), and ethics problems (such as human embryo editing risks).Future efforts should be placed on refining editing tools (e.g. Cas12a and base editing to minimise the off-target effect), innovating delivery systems (exosomes, smart nanoparticles for better targeting), designing multi-drug combination therapy (CRISPR + CAR-T to tackle heterogeneity), and investigation in non-human primates safety aspects (long-term study).There needs to be interdisciplinary cooperation if these technologies need to be disseminated from research labs to clinical practice more quickly.

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