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From Bench to Bedside: Multidimensional Clinical Applications and Challenges of CRISPR Genome Editing

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Abstract

CRISPR-Cas9 gene editing has shifted from the laboratory to clinical application in specific diseases, where, in β-hemoglobinopathies, disrupting the BCL11A erythroid enhancer can reactivate fetal hemoglobin, functionally curing transfusion-dependent β-thalassemia and sickle cell disease, ultimately giving birth to Casgevy™, the first approved CRISPR therapy. In oncology field, CRISPR-engineered T cells precisely insert CARs into TRAC locus or knock out immune checkpoints such as PD-1, thereby showing enhanced persistence, reduced exhaustion and high response rates in hematologic malignancies such as multiple myeloma, while in solid tumors, α-thalassemia due to large fragment deletions of HBA1/HBA2, hemophilia A limited by F8 cDNA size exceeding viral packaging capacity and acute respiratory viral infections such as SARS-CoV-2 and influenza, significant obstacles still exist. These challenges include inefficient delivery, lack of physiological bypass mechanisms, rapid viral mutation, and a narrow therapeutic window; furthermore, Casgevy™'s cost of up to 2.2 million dollars highlights a critical accessibility gap, with use limited to affluent healthcare systems. Emerging solutions include double-strand break-free base editors and prime editors, in vivo delivery via lipid nanoparticles, and multimodal therapeutic regimens; however, without synchronous progress in equitable pricing and global accessibility, CRISPR's curative potential will remain unrealized for the majority of patients worldwide.

Keywords

CRISPR-CAS9, T-cell, gene therapy, hematological genetic diseases, antiviral immunity

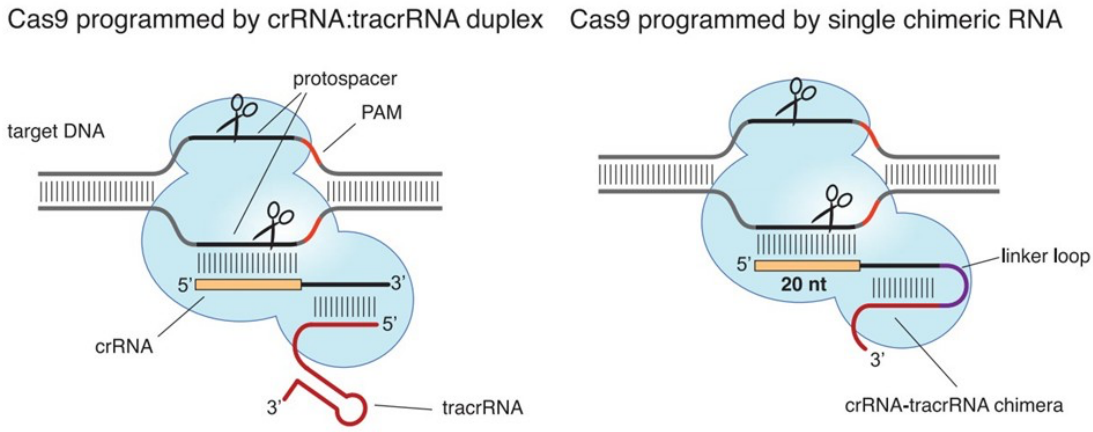
1. Introduction

CRISPR was originally an adaptive immune system of bacteria and archaea: it captures viral or plasmid DNA fragments as "spacer sequences". It inserts them into their own genomes, surrounded by repetitive sequences on both sides. When reinfected, crRNA is transcribed and generated, which binds to Cas proteins to precisely recognize and cut invasive nucleic acids. This system consists of three stages: adaptation (acquisition interval), expression (crRNA generation), and interference (target degradation). It is a survival mechanism evolved by microorganisms in their long-term battle against viruses, long before humans used it as a gene editing tool.

The programmable application of this system was first demonstrated in 2012 by Jinek et al., who reconstituted the Streptococcus pyogenes CRISPR-Cas9 mechanism in vitro [1]. This engineered platform relies on a chimeric single-guide RNA (sgRNA)—a synthetic fusion of crRNA and tracrRNA—to direct Cas9 endonuclease to any genomic locus adjacent to a 5'-NGG-3' protospacer adjacent motif (PAM), whereupon binding, Cas9 induces blunt-ended double-strand break (DSB) through its HNH (complementary strand) and RuvC-like (non-complementary strand) nuclease domains. Cellular repair subsequently proceeds through one of two endogenous pathways: error-prone non-homologous end joining (NHEJ), which frequently generates insertions or deletions leading to frameshift-mediated gene knockout, or homology-directed repair (HDR), which enables precise nucleotide substitution when co-delivered with donor template, with the efficiency of HDR remaining low in quiescent cells such as hematopoietic stem cells.

The transformative potential of CRISPR lies not only in the precision of its targeting, but more in its ability to intervene at the root of disease—correcting mutations of single genes, reprogramming specificity of T cells, or degrading pathogenic RNA genomes. At the same time, unlike conventional drug therapies that modulate downstream phenotypes, it offers the prospect of definitive, single-dose cure. However, this prospect is conditional, as its clinical efficacy depends not merely on the efficiency of editing, but more on the permissiveness of the disease context: is the target accessible? Do bypass pathways exist functionally? Can edited cells engraft and persist? These biological constraints—rather than technical limitations—now define the frontier of therapy.

*Figure 1: Two Design Approaches of Guide RNA for the CRISPR-Cas9 Gene Editing System[1]**Martin et al. Science. 2012 AUG;337***



2. CRISPR-Edited T Cells

2.1 CRISPR-Engineered T Cell Therapies in Oncology

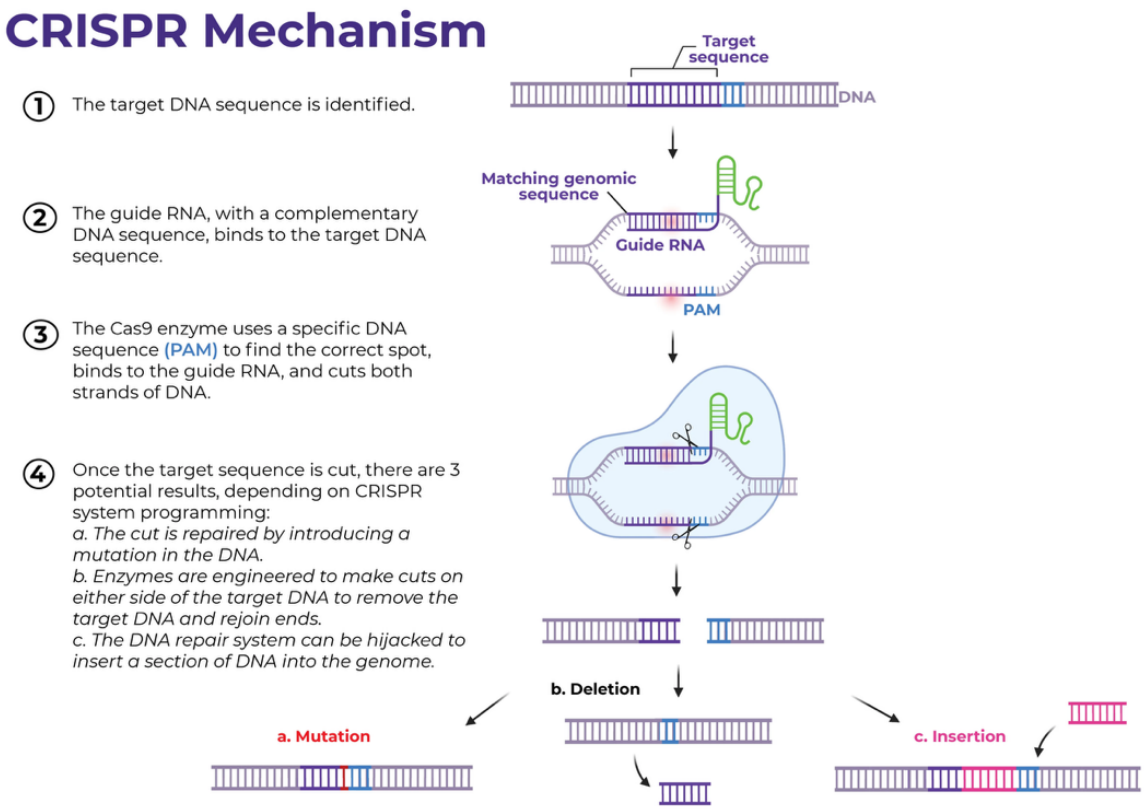
First clinical validation of multiplex CRISPR editing in human body came from Stadtmauer et al. [2], where three patients with advanced malignant tumors—two multiple myeloma patients, one synovial sarcoma patient—received autologous T cells edited at three loci: TRAC and TCRβ were disrupted through NHEJ to eliminate endogenous TCR expression, and NY-ESO-1-specific transgenic TCR was inserted through HDR. Edited cells persisted for more than 9 months without severe cytokine release syndrome, immune effector cell–associated neurotoxicity syndrome (ICANS) or oligoclonal expansion, although only one patient showed transient tumor regression, with the true significance of this trial lying in demonstrating that human T lymphocytes can tolerate triple genomic editing without losing viability or inducing genomic catastrophe—a long-standing concern in adoptive cell therapy.

2.2 Further Optimization Using CRISPR to Enhance CAR-T Efficacy

Eyquem et al. advanced this paradigm by redesigning the delivery of CAR itself, and they did not rely on random lentiviral integration—which leads to variability in transgene expression and susceptibility to epigenetic silencing—but instead used CRISPR to precisely knock CD19-specific CAR into the TRAC locus

[3]. This generated uniform, stable CAR expression under the control of an endogenous TCR promoter, while simultaneously disrupting the native TCR, thereby eliminating allogeneic reactivity and enabling the development of allogeneic ("off-the-shelf") products. Most critically, these TRAC-CAR T cells retained stem cell memory-like (TSCM) phenotype, which was associated with enhanced metabolic fitness, reduced exhaustion markers (PD-1, LAG-3, TIM-3), and prolonged persistence, and in xenograft models of B-cell acute lymphoblastic leukemia (B-ALL), they conferred 100% long-term survival—significantly superior to conventionally engineered CAR-T cells. This was not incremental optimization, but a foundational redesign of the cell product. architecture.

Figure 2: Flowchart of CRSIPR gene editing technology [4]



2.3 CRISPR Clinical Practice in Multiple Myeloma (MM) and Neuroblastoma

This logic has since been extended to other hematologic malignancies. In multiple myeloma, the bone marrow microenvironment exerts profound immunosuppressive pressure through TGF-β, adenosine, and PD-L1, while combining BCMA-targeted CAR-T therapy with PD-1 knockout produced an overall response rate exceeding 90% in a pivotal trial of ciltacabtagene autoleucel. This dual strategy enhances antigen recognition while releasing checkpoint inhibition, thereby directly implementing principles originally validated in the Stadtmauer feasibility study.

Solid tumors remain difficult to treat. In neuroblastoma, although GD2-directed CAR-T cells show encouraging data in early stages, clinical responses are limited by dense stromal barriers, hypoxia, nutrient deprivation, and heterogeneous antigen expression, while CRISPR has been used to disrupt inhibitory receptors, such as PD-1 and TGFβR2, and to perform in situ editing of tumor cells. Zhang et al. [5] used intratumoral AAV delivery to knock CXCL10 into neuroblastoma cells, thereby creating a chemotactic gradient that enhances CAR-T infiltration; however, physical exclusion, metabolic competition, and rapid antigen loss continue to undermine efficacy. It is increasingly clear that CRISPR alone cannot dismantle the multifactorial defenses of the solid tumor microenvironment; combinatorial approaches, oncolytic virus therapy, metabolic modulators, and cytokine armoring are essential.

2.4 Existing Problems and Future Prospects of CRISPR in Anti-Cancer Therapy

2.4.1 Current Challenges

Safety concerns remain. A landmark study revealed that CRISPR-induced DSBs can trigger kilobase-scale deletions, inversions, or chromothripsis, which are potentially oncogenic if these structural variations disrupt tumor suppressor genes like TP53 or PTEN (Nat Biotechnol 2022;40:107–117), while manufacturing logistics further complicate challenges: autologous CAR-T therapy costs >$300,000 and requires 3–5 weeks of ex vivo expansion time, thereby excluding patients with aggressive diseases. Allogeneic alternatives shorten turnaround time, but often exhibit poor in vivo persistence due to host-mediated rejection, and although short-term follow-up shows no clonal advantage, long-term risk of edited hematopoietic clones leading to secondary malignancies remains unknown, which requires monitoring beyond scope of current trials.

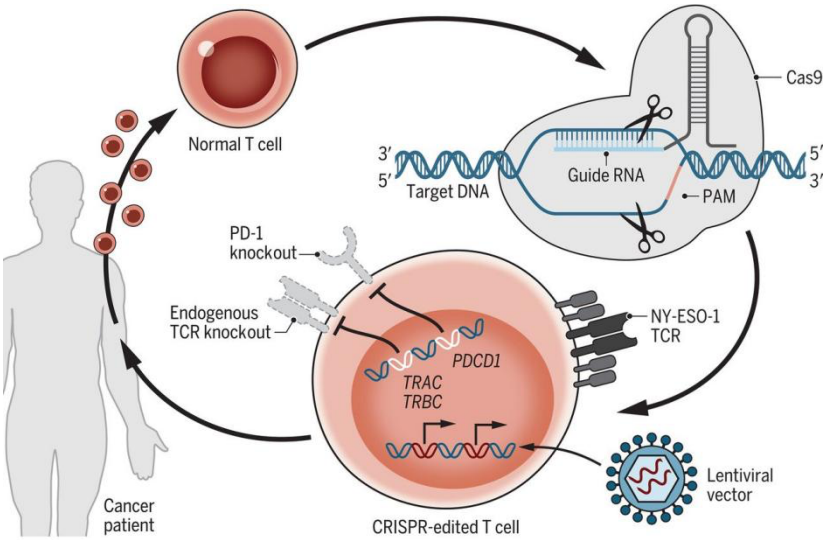
2.5 Future Outlook

Future may lie in fusion, where base and prime editors reduce genetic toxicity risk by catalyzing C•G-to-T•A or small fragment insertions/deletions without producing DSBs, while machine learning models trained on single-cell multi-omics maps of tumor-immune interactions can identify new targets beyond classical checkpoints. Most promising is in vivo CAR-T generation, as Rurik et al. [6] demonstrated that lipid nanoparticles (LNPs) encoding Cas9 mRNA and sgRNA can directly reprogram endogenous T cells within mouse host body, thereby completely bypassing leukapheresis and ex vivo culture. When integrated with mRNA vaccines to enhance antigen presentation, or integrated with oncolytic viruses to convert “cold” tumors into “hot” tumors, such platforms can achieve scalable, off-the-shelf cell therapy.

3. CRISPR Editing for Blood Genetic Diseases

Therapeutic potential of CRISPR has achieved most decisive realization in β-hemoglobinopathies, where Frangoul et al. [7] reported results of CLIMB THAL-111 and CLIMB SCD-121 trials, and in these trials, patients with transfusion-dependent β-thalassemia or severe sickle cell disease (SCD) received exagamglogene autotemcel (exa-cel) treatment. $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) were edited ex vivo to disrupt +58 erythroid-specific enhancer of BCL11A, which is a transcriptional repressor that silences γ-globin after birth, and by relieving this repression, production of fetal hemoglobin (HbF) was reactivated, thereby compensating for defective adult β-globin. Two index patients both achieved transfusion independence or freedom from vaso-occlusive crises, with HbF levels exceeding >40%, while whole-genome sequencing detected no off-target edits above background level, and polyclonal engraftment persisted >24 months, thus leading to regulatory approval of Casgevy™ in 2023, the first CRISPR-based therapy. therapeutic.

Figure 3: CRISPR-Cas9 technology is used to modify T cells for the treatment of β-thalassemia and sickle cell anemia [8].



3.1 CRISPR Editing for β-Thalassemia & Sickle Cell Disease

3.1.1 Two Strategies in β-hemoglobinopathy Treatment

CRISPR gene editing therapy and traditional lentiviral gene therapy represent two different strategies in the treatment of β -hemoglobinopathy. Lentivirus therapy provides functional supplementation by randomly integrating vectors carrying normal β-globin genes, but it carries the risk of insertional mutations and is complex to manufacture and costly. In contrast, CRISPR therapy does not repair the mutated HBB gene but precisely edits the BCL11A enhancer to reactivate fetal hemoglobin, utilizing the natural pathways already present during development to achieve treatment. DeWitt et al. (Nat Med 2016; 22:1343-1348) [9] proved for the first time that this editing did not affect the multiline differentiation ability of HSPCs. Wu et al. (Nat Med 2019; [10] almost complete elimination of sickle cell disease was observed in sickle cell disease models, while two recent Phase III studies (Cappellini n=42;) Esrick n=31) showed that over 90% of patients had no disease manifestations at 24 months and no evidence of clonal hematopoiesis (Blood Adv 2023;7: 4323-4334; n=42) [11] and Esrick (Blood Adv 2023; Morning 35-4346; n=31) [12]. This strategy avoids the risk of exogenous gene integration. Its ingenuity lies in "borrowing" the naturally evolved compensation mechanism rather than forcibly correcting genetic defects.

3.1.2 Preclinical Exploration and Fundamental Limitations in α-Thalassemia and Hemophilia A

This strategy will fail in the absence of such redundancy. In α-thalassemia, severe forms (for example, Hb Bart's hydrops fetalis) typically involve large fragment deletions containing HBA1 and HBA2 genes—leaving no template for repair, while Liu et al. [13] used adenine base editors to restore minimal α-globin expression in rare non-deletion point mutation cases, but this approach excludes >95% of patients. Attempting to elevate HbF as explored by Traxler et al. is futile [14]: HbF (α2γ2) still requires α-chains for tetramer assembly, without which, γ-globin accumulates as toxic aggregates.

Hemophilia A presents a unique obstacle, as F8 cDNA (~7 kb) exceeds packaging capacity of adeno-associated virus vector (~4.7 kb). Wang et al. [15] employed dual AAVs encoding split F8 halves linked by inteins, relying on post-translational splicing, but co-transduction efficiency in hepatocytes was very low, and anti-AAV immunity restricted re-administration. Yin et al. [16] bypassed AAV by using LNPs to deliver CRISPR components to achieve targeted integration of B-domain-deleted F8 into the albumin locus, and while this avoided capsid-associated toxicity, FVIII expression remained below a therapeutic level (<12% of normal), with it being highly variable between subjects. Without a physiological bypass similar to HbF, these monogenic diseases remain confined to preclinical exploration.

3.1.3 Challenges and Limitations

Then there is the problem of cost. Casgevy™ is priced at $2.2 million per patient, which makes it unaffordable for health systems in almost all countries outside North America and Western Europe. While short-term safety seems to be performing well, delayed genotoxicity, such as chromothripsis or insertional mutagenesis activating proto-oncogenes, cannot be ruled out without decades of monitoring. The paradox is stark: a functional cure already exists, but global accessibility is minimal.

4. CRISPR Editing for Respiratory Viral Infections

4.1 CRISPR Anti-SARS-CoV-2 Research Progress

Currently, CRISPR Anti-SARS-CoV-2 is at the forefront of clinical trials. We will use cas13, and this is not a dna editing technology but an rna editing fund. For RNA viruses, relevant effector is Cas13, not Cas9, while Gootenberg's [17] SHERLOCK platform utilizes collateral RNase activity of Cas13 for signal amplification, which was rapidly adapted for SARS-CoV-2 detection, with its sensitivity being comparable to RT-PCR. In terms of treatment, Abdelrahman et al. proposed PAC-MAN [18]: Cas13d guided by crRNAs targeting conserved regions reduced viral RNA of different variants by more than 90% in cultures of human airway epithelium, but problems such as efficient delivery to respiratory epithelium, avoiding activation of innate immunity, and preventing viral escape through point mutations remain unresolved.

4.2 CRISPR Anti-Influenza Virus Research Progress

Similar strategies have also been targeted against influenza. Chen et al. [19] used SHERLOCK to rapidly classify subtypes of H1N1, H3N2 and influenza B. Abdelrahman et al. [20] delivered Cas13d mRNA to H1N1-infected mice through aerosolized LNPs, thereby achieving a 100-fold reduction in lung viral titer and increasing survival rate from 20% to 80%, but despite these advances, the high mutation rate of influenza makes crRNAs obsolete within days. Furthermore, unlike in chronic diseases where edited cells can persist, antiviral CRISPR must function within a narrow therapeutic window, ideally before symptom onset, while avoiding bystander cleavage of host transcripts.

4.3 Challenges and Shortcomings

Crucially, there are currently no human trials evaluating CRISPR as antiviral therapeutic approach, as obstacles include poor biodistribution of LNPs to distal airways, kinetics of transient expression, and toxicity inherent to promiscuous RNA degradation, and for acute infections, the threshold for clinical translation is exceptionally high.

5. Current Clinical Landscape

Overall, these advances sketch out a distinctly bifurcated clinical landscape for CRISPR, where in hematologic malignancies and β-hemoglobinopathies, technology has crossed from experimental intervention to threshold of approved therapy, thereby bringing durable remission and functional cure. In contrast, solid tumors, α-thalassemia, hemophilia A and acute respiratory viral infections remain formidable frontiers, constrained not by lack of creativity but by fundamental biological or logistical barriers. This pattern is consistent, as CRISPR excels when the disease mechanism is monogenic, the cellular substrate is amenable to ex vivo editing, and a physiological workaround exists (e.g., HbF reactivation, TCR locus hijacking), while it encounters difficulty when the target is deleted, oversized, embedded in an immunosuppressive microenvironment, or transiently present.

6. Discussion

Disease-dependent efficacy of this kind challenges concept of CRISPR as universal molecular scalpel, as it reflects permissive biological characteristics in liquid tumors and β-hemoglobinopathies: accessible cell types (T cells, HSPCs), clearly defined genetic drivers, and exploitable redundancy. In contrast, solid tumors operate in chaotic, dynamic ecosystems, where antigen heterogeneity, stromal exclusion, and metabolic antagonism render single-gene editing insufficient to cope, while similarly, in α-thalassemia, there is no switch to flip—only missing hardware, and Hemophilia A is constrained by vector physics rather than editing fidelity. For acute viral infections, mismatch between CRISPR kinetics and clinical reality—patients present too late, delivery efficiency is low, and collateral activity of Cas13 brings unacceptable risks—leaves all data in preclinical stage.

Equally important is gap in equity, as approved therapies cost hundreds of thousands to millions of dollars, thereby excluding vast majority of global population from access. Autologous manufacturing is inherently non-scalable by nature, while allogeneic and in vivo approaches offer theoretical solutions but still lag behind in terms of durability or safety. Furthermore, while short-term genotoxicity appears to be low, potential delayed chromosomal shattering or clonal evolution requires decades of follow-up, and we currently do not possess this data, which means the field must strike balance between optimism and caution, and balance between innovation and accessibility.

7. Conclusion

CRISPR has undeniably reshaped the therapeutic paradigm for select genetic and hematologic disorders, transforming once-fatal conditions into manageable—or even curable—diseases. Yet its journey is far from complete. The next decade will test whether it can transcend its current niches through safer editors (base/prime), smarter delivery (LNPs, AAV capsid engineering), and integrated multimodal regimens. But technical advancement alone is insufficient. True impact requires confronting the ethical and economic barriers

that separate discovery from delivery. Only then can CRISPR fulfill not just its scientific promise, but its humanitarian one.

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Conflicts of Interest

The authors declare no conflict of interest.

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