# CRISPR-Cas9: Opportunities and Challenges in the Therapeutic Landscape of Leukemia

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***Abstract*** **- Leukemia, a heterogeneous group of hematologic malignancies originating from aberrant proliferation of hematopoietic stem and progenitor cells, remains one of the leading causes of cancer-related morbidity and mortality worldwide. Despite significant progress in chemotherapy, radiotherapy, hematopoietic stem cell transplantation (HSCT), and immunotherapeutic approaches, disease relapse and therapy resistance continue to present major obstacles to long-term remission. The emergence of the clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9 (Cas9) system has revolutionized genome editing, offering a simple, precise, and programmable tool for targeted gene manipulation.**

**CRISPR-Cas9 has been applied extensively to dissect leukemogenic pathways, identify novel oncogenic drivers, and develop innovative therapeutic strategies, including gene correction, immunotherapy enhancement, and synthetic lethality screening. However, its translation into clinical oncology remains challenged by off-target mutagenesis, delivery inefficiencies, ethical considerations, and regulatory barriers. This review critically examines the mechanistic principles of CRISPR-Cas9, its therapeutic potential in leukemia, and the evolving landscape of CRISPR-based clinical research, while highlighting future prospects such as base editing, prime editing, and Cas variants that may improve safety and precision.**

***Keywords*** **- CRISPR-Cas9; Leukemia; Genome editing; Immunotherapy; Gene correction; Off-target effects; Cancer therapeutics**

## 1. INTRODUCTION

Leukemia comprises a diverse set of hematologic malignancies that originate from clonal expansion and impaired differentiation of hematopoietic stem and progenitor cells [1]. It is broadly classified into four major subtypes — acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), and chronic myeloid leukemia (CML) — each defined by specific cytogenetic and molecular characteristics [2]. According to the Global Cancer Observatory (GLOBOCAN 2024), leukemia accounted for approximately 4, 80,000 new cases and 3, 10,000 deaths worldwide, representing 2.5% of all new cancer diagnoses [3]. Although therapeutic advancements have improved survival in certain subtypes, such as pediatric ALL and CML, other forms, particularly relapsed AML, remain difficult to cure due to genetic heterogeneity, drug resistance, and clonal evolution [4, 5].

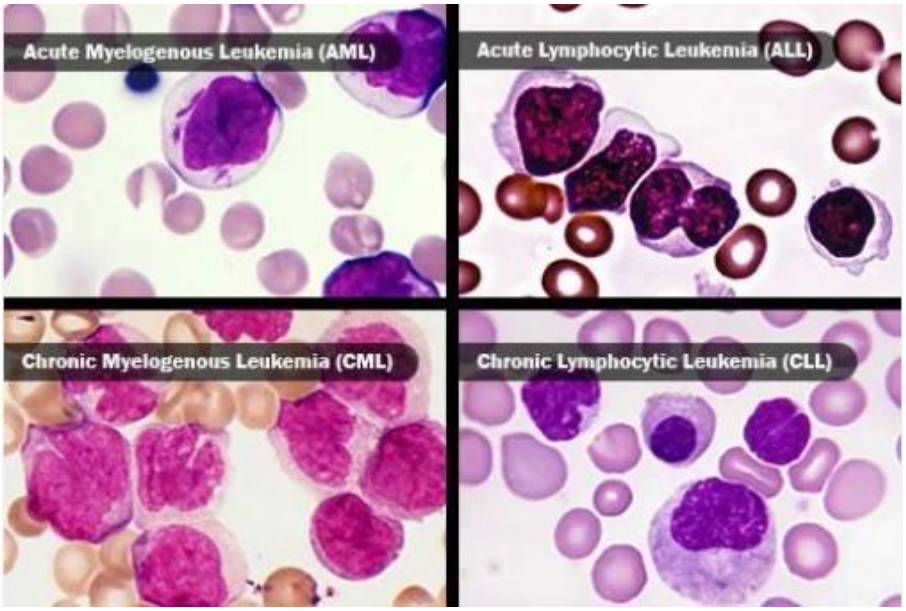


Fig. 1: Types of Leukemia [6]

### *1.1 Current therapeutic landscape and limitations*

Conventional leukemia therapies, including cytotoxic chemotherapy and radiotherapy, have achieved temporary remission in many cases but often result in high relapse rates and severe systemic toxicity [7]. Hematopoietic stem cell transplantation (HSCT) provides curative potential for select patients; however, its success is limited by graft-versus-host disease (GVHD), infection risks, and donor unavailability [8]. The introduction of targeted therapies such as imatinib for CML and monoclonal antibodies like blinatumomab for B-cell ALL has transformed treatment paradigms [9, 10]. Yet, secondary resistance mutations and clonal escape frequently emerge, reducing long-term efficacy [11]. Hence, precision therapies that target the root molecular causes of leukemogenesis, while minimizing collateral toxicity, are urgently required.

### *1.2 The advent of gene-editing technologies*

The idea of targeted genome manipulation has evolved through several technological milestones. Zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) were among the earliest programmable nucleases enabling site-specific DNA cleavage [12]. Despite their precision, these tools required complex protein engineering, limiting scalability and accessibility [13]. The discovery of the CRISPR-Cas9 system in Streptococcus pyogenes, an adaptive immune mechanism against bacteriophage infection, represented a paradigm shift in genome engineering [14]. Unlike ZFNs and TALENs, CRISPR-Cas9 uses a short guide RNA (sgRNA) to direct the Cas9 nuclease to complementary genomic sequences, generating double-strand breaks that can be repaired by non-homologous end joining (NHEJ) or homology-directed repair (HDR) [15].

This RNA-guided editing mechanism offers unmatched simplicity, efficiency, and versatility, facilitating widespread use in functional genomics, disease modeling, and therapeutic development [16]. Since its adaptation for eukaryotic gene editing in 2013, CRISPR-Cas9 has been adopted in thousands of studies across diverse biological systems, including human hematopoietic cells [17].

### *1.3 CRISPR-Cas9 and its relevance to leukemia*

Leukemia, being driven by well-characterized genetic mutations, presents an ideal model for CRISPR-Cas9–based research and therapy [18]. Genome-wide CRISPR knockout screens have been instrumental in identifying essential genes for leukemic cell survival, drug resistance, and immune evasion [19]. Furthermore, CRISPR-Cas9 enables correction of driver mutations such as FLT3-ITD, NPM1c, or TP53, which play critical roles in AML progression [20, 21]. In immunotherapy, CRISPR technology enhances the efficacy of chimeric antigen receptor (CAR)-T cells by knocking out immune checkpoint regulators like PD-1 or CTLA4, thereby strengthening anti-tumor activity [22].

CRISPR-mediated editing of hematopoietic stem cells also holds potential for durable remission by reprogramming the leukemic genome or introducing tumor-suppressive genes ex vivo before transplantation [23]. Early preclinical studies have shown promising results, with CRISPR-edited cells demonstrating selective eradication of leukemic clones in mouse xenograft models [24].

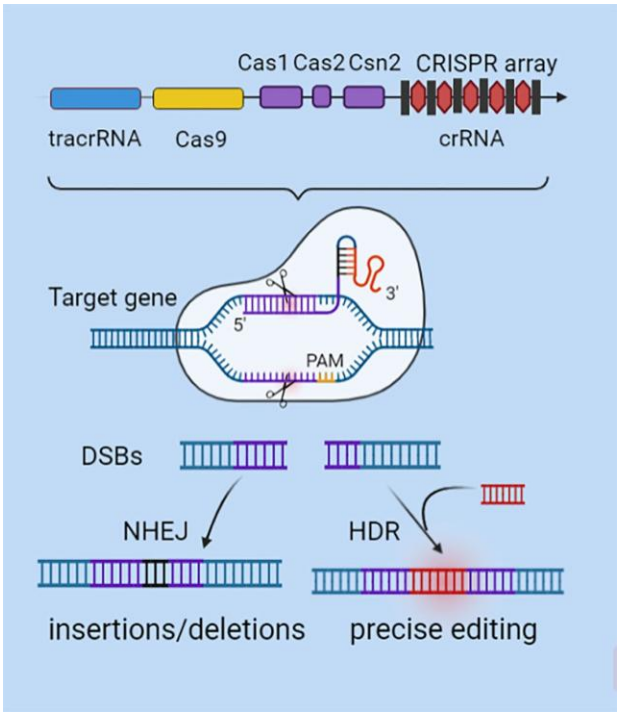


Fig. 2: Overview of CRISPR-Cas9 [25]

## 2. CRISPR-Cas9 TECHNOLOGY: MECHANISMS AND EVOLUTION

### *2.1 Structure and mechanism of Cas9-mediated genome editing*

Cas9 is a large multidomain endonuclease composed of two major lobes: the recognition (REC) lobe and the nuclease (NUC) lobe [26]. The REC lobe, including the Rec I, Rec II, and bridge helix domains, mediates RNA and DNA binding, while the NUC lobe contains the RuvC and HNH catalytic domains responsible for double-strand DNA cleavage [27]. Additionally, the PAM-interacting (PI) domain within Cas9 recognizes the protospacer adjacent motif (PAM), which for S. pyogenes Cas9 (SpCas9) is typically 5′–NGG–3′ [28].

The CRISPR-Cas9 editing process involves several sequential steps:

1. sgRNA loading: The Cas9 protein forms a ribonucleoprotein complex with a single-guide RNA (sgRNA), which fuses the crRNA (target-specific) and tracrRNA (scaffold) into a single molecule [29].

2. Target DNA recognition: The sgRNA directs Cas9 to a complementary DNA sequence adjacent to a PAM site. PAM recognition induces local DNA melting and hybridization with the sgRNA's seed region [30].

3. DNA cleavage: Cas9 undergoes conformational activation, allowing the HNH domain to cleave the complementary strand and the RuvC domain to cleave the non-complementary strand, resulting in a blunt-ended double-strand break [31].

4. Repair pathway engagement: The cell's endogenous repair machinery then resolves the break via non-homologous end joining (NHEJ) or homology-directed repair (HDR) [32].

NHEJ frequently produces small insertions or deletions (indels), leading to gene disruption, whereas HDR enables precise genome correction if a donor DNA template is supplied [33]. The balance between these repair mechanisms depends on the cell cycle stage and is a critical determinant of editing outcome [34].

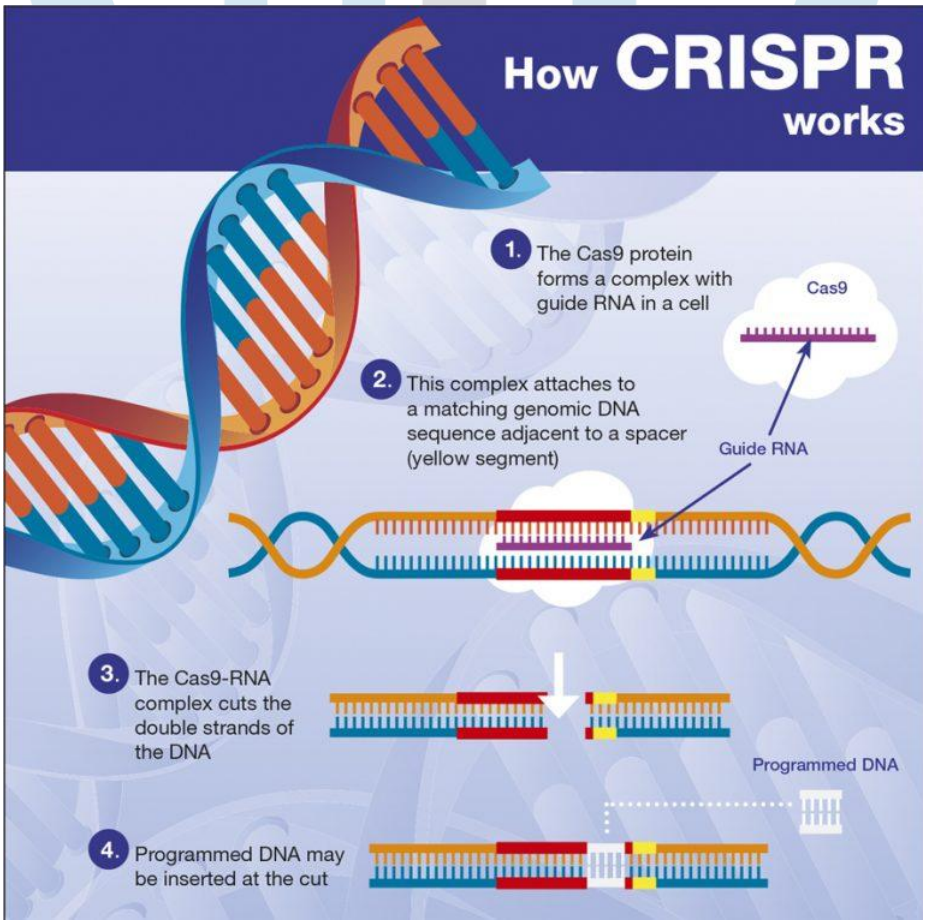


Fig. 3: Mechanism of CRISPR-Cas9 [35]

### *2.2 Guide RNA design and target specificity*

Guide RNA (gRNA) design is a major determinant of CRISPR efficiency and specificity. The 20-nucleotide spacer sequence must be complementary to the target DNA adjacent to a PAM site. However, mismatches, particularly within the 8–12 bp seed region, can lead to reduced activity or off-target cleavage [36].Incorporating high-fidelity Cas variants with optimized sgRNA design has significantly improved genome-editing precision in hematopoietic and leukemic models [37].

### *2.3 Delivery systems for CRISPR-Cas9 in hematologic applications*

Efficient and safe delivery of CRISPR components remains one of the greatest challenges in clinical translation, particularly in hematologic malignancies. Various delivery modalities have been developed, broadly categorized into viral and non-viral systems.

Viral delivery systems such as lentiviruses and adenoviruses allow stable integration or transient expression of Cas9 and sgRNA in dividing and non-dividing cells [38]. Lentiviral systems are widely used for functional genomic screening in leukemia cell lines due to their high transduction efficiency [39]. However, integration-associated mutagenesis and immunogenicity pose significant risks for therapeutic applications [40].

Adeno-associated viruses (AAVs), especially serotype 6 (AAV6), have demonstrated high tropism for hematopoietic stem cells (HSCs), making them suitable for ex vivo gene correction approaches [41]. Nevertheless, their limited packaging capacity (~4.7 kb) restricts the use of large Cas9 constructs such as SpCas9, necessitating the adoption of compact variants like SaCas9 [42].

Non-viral delivery methods, including lipid nanoparticles (LNPs), electroporation, and ribonucleoprotein (RNP) complexes, have gained traction for transient Cas9 delivery. These strategies avoid genomic integration and reduce immunogenicity [43]. RNP complexes, in particular, provide rapid, high-efficiency editing in primary hematopoietic cells with minimal off-target effects, representing a major step toward clinically viable CRISPR therapeutics [44].Emerging technologies such as cell-penetrating peptides, gold nanoparticles, and exosome-based delivery are being explored to further enhance hematopoietic targeting and systemic safety [45].

## 3. APPLICATION OF CRISPR–Cas9 IN LEUKEMIA RESEARCH AND THERAPY

### *3.1 Functional Genomic Screening in Leukemia*

The advent of CRISPR–Cas9 has revolutionized functional genomics by allowing systematic interrogation of gene function through genome-wide loss-of-function screens [46]. In leukemia, this approach has enabled identification of novel oncogenic drivers, resistance mechanisms, and therapeutic vulnerabilities [47].

Tzelepis et al. conducted one of the earliest large-scale CRISPR knockout screens in acute myeloid leukemia (AML) cell lines, revealing essential genes within the Wnt, MYC, and MAPK pathways [48]. Similarly, Wang et al. performed genome-wide CRISPR screens in B-cell acute lymphoblastic leukemia (B-ALL), identifying PAX5, RUNX1, and IKZF1 as lineage-essential regulators [49]. These studies underscore the potential of CRISPR-based screening in uncovering critical dependencies that can be therapeutically exploited.

Beyond identifying essential genes, CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) systems enable transcriptional modulation without inducing double-strand breaks [50]. By coupling a catalytically inactive Cas9 (dCas9) to transcriptional repressors or activators, researchers can dissect enhancer landscapes and regulatory networks governing leukemogenesis [51]. For example, Fulco et al. applied CRISPRi to map enhancer–gene interactions in leukemia cells, revealing distal regulatory elements crucial for MYC expression [52].

### *3.2 Correction of Oncogenic Mutations*

One of the most promising applications of CRISPR–Cas9 in leukemia involves gene correction of disease-causing mutations. Mutations in genes such as FLT3, NPM1, IDH1/2, and RUNX1 are common in AML and can serve as targets for precise gene editing [53].

In 2017, Heckl et al. demonstrated the correction of FLT3 internal tandem duplication (FLT3-ITD) mutations using CRISPR–Cas9 combined with homology-directed repair (HDR), restoring normal signaling in AML models [54]. Similarly, CRISPR-mediated editing of NPM1c+ mutations reversed aberrant cytoplasmic localization of nucleophosmin, inducing differentiation in leukemic blasts [55].

CRISPR has also been applied to model specific leukemia-associated translocations. Torres et al. successfully recapitulated the t (8; 21) (q22; q22) RUNX1–RUNX1T1 fusion and inv (16) (p13q22) CBFB–MYH11 rearrangements in hematopoietic progenitor cells, generating accurate de novo models of AML [56]. Such CRISPR-generated models facilitate mechanistic exploration of oncogenesis and therapeutic response testing [57].

### *3.3 Dissecting Mechanisms of Drug Resistance*

Resistance to chemotherapy and targeted therapies remains a critical obstacle in leukemia treatment. CRISPR screens have been pivotal in elucidating molecular mechanisms of drug resistance [58]. Chen et al. identified loss-of-function mutations in TP53, KEAP1, and CUL3 that confer resistance to FLT3 inhibitors in AML through genome-wide CRISPR dropout assays [59].

In CML, CRISPR-Cas9–mediated mutagenesis of BCR-ABL1 revealed secondary resistance mutations including T315I, which diminish TKI binding affinity [60]. Moreover, CRISPR-based editing has been leveraged to create drug-resistant leukemia models, enabling the preclinical evaluation of next-generation inhibitors [61].

CRISPR also provides insight into immunotherapy resistance mechanisms. For example, Shifrut et al. used pooled CRISPR screens in T cells to uncover regulators of CAR-T cell exhaustion, identifying the transcription factor NR4A3 as a target for enhancing antitumor efficacy [62].

### *3.4 Engineering Immune Cells for Leukemia Therapy*

A transformative application of CRISPR–Cas9 lies in engineering immune effector cells for leukemia immunotherapy [63]. The most notable example involves the generation of chimeric antigen receptor (CAR) T cells, in which autologous T cells are modified to express synthetic receptors targeting leukemic antigens such as CD19 or CD22 [64].

Traditional viral vector–based approaches for CAR insertion pose challenges including insertional mutagenesis and manufacturing complexity [65]. CRISPR-based methods allow precise integration of CAR constructs into safe genomic loci, such as the TRAC (T-cell receptor alpha constant) locus, ensuring uniform CAR expression and reducing tonic signaling [66].

Ren et al. demonstrated that CRISPR-mediated TRAC disruption combined with CAR insertion significantly enhanced T-cell persistence and antitumor activity in preclinical leukemia models [67]. Additionally, disruption of immune checkpoint genes such as PD-1, LAG-3, and CTLA-4 via CRISPR editing has been shown to augment CAR-T cytotoxicity and persistence [68].

Beyond CAR-T cells, CRISPR has also been used to enhance natural killer (NK) cells for leukemia immunotherapy. CRISPR knockout of CISH, a negative regulator of cytokine signaling, potentiates NK cell–mediated cytotoxicity against AML targets [69]. These strategies exemplify the merging of genome editing and cell-based therapy for precision leukemia treatment [70].

### *3.5 CRISPR for Hematopoietic Stem Cell (HSC) Engineering*

The ability to edit hematopoietic stem and progenitor cells (HSPCs) ex vivo represents a major leap toward curative leukemia therapies [71]. HSCs serve as an ideal target due to their self-renewal capacity and multilineage potential.

Dever et al. successfully corrected the sickle-cell disease mutation in HSCs using CRISPR–Cas9, providing a proof-of-concept for gene correction in hematologic disorders [72]. This platform is being adapted to leukemia for correcting preleukemic mutations or introducing protective alleles [73].

One challenge in HSC editing is efficient delivery of CRISPR components. Recent advances using ribonucleoprotein (RNP) complexes and electroporation have improved editing efficiency while minimizing genotoxicity [74]. Moreover, integrating CRISPR editing with base editing or prime editing systems could enable scarless correction of single-nucleotide variants associated with leukemia predisposition [75].

### *3.6 Diagnostic and Predictive Applications*

CRISPR-based platforms have also emerged as diagnostic tools for leukemia detection and molecular monitoring [76]. Systems like SHERLOCK (Specific High-sensitivity Enzymatic Reporter UnLOCKing) and DETECTR utilize Cas enzymes (Cas12, Cas13) to identify specific nucleic acid sequences with high sensitivity [77].

For example, CRISPR-based assays have been developed to detect BCR-ABL1 fusion transcripts, allowing real-time minimal residual disease (MRD) monitoring with single-molecule precision [78]. These approaches offer rapid, cost-effective alternatives to PCR and next-generation sequencing for early detection and relapse prediction [79].

### *3.7 Translational Potential and Preclinical Advances*

Multiple preclinical studies demonstrate that CRISPR-edited cells retain functionality and safety, supporting progression toward clinical translation [80]. For instance, Xu et al. reported that CRISPR-corrected HSPCs engrafted successfully in murine bone marrow without tumorigenic transformation [81]. Early-phase clinical trials (e.g., NCT03747965) have explored CRISPR-edited T cells for relapsed leukemia, showing encouraging preliminary safety outcomes [82].

However, translational hurdles persist, including immune rejection of edited cells, off-target mutagenesis, and regulatory complexities surrounding germline editing [83]. Nevertheless, the convergence of CRISPR technology, stem-cell engineering, and immunotherapy is poised to redefine leukemia management [84].

## 4. OPPORTUNITIES — ADVANCES IN CRISPR-BASED LEUKEMIA TREATMENTS

### *4.1 Precision Targeting and Personalized Therapy*

CRISPR–Cas9 technology offers a unique opportunity to develop personalized therapies tailored to a patient's specific mutational landscape [85]. Unlike traditional chemotherapy, which broadly targets proliferating cells, CRISPR enables the correction or knockout of precise oncogenic mutations responsible for leukemic transformation [86].

For example, personalized CRISPR editing could directly correct FLT3-ITD, IDH2-R140Q, or RUNX1 mutations identified through patient sequencing, restoring normal hematopoiesis [87]. This precision targeting minimizes systemic toxicity and off-target damage—one of the major limitations of current AML therapies [88].

Moreover, combining CRISPR-based gene correction with high-throughput genomic profiling allows clinicians to design individualized therapeutic regimens, predicting patient-specific responses to targeted or immune-based treatments [89]. Such strategies align with the principles of precision oncology, enabling a shift from generic protocols to mutation-informed, cell-based therapies [90].

### *4.2 Combination of CRISPR with Emerging Technologies*

Integration of CRISPR–Cas9 with other innovative biotechnologies has unlocked new therapeutic possibilities [91]. The coupling of CRISPR with single-cell sequencing (scRNA-seq) enables detailed mapping of transcriptional responses following genetic perturbations, revealing heterogeneity in leukemia cell populations [92].

CRISPR has also been synergized with epigenome-editing platforms, allowing modulation of DNA methylation and histone modification landscapes associated with leukemogenesis [93]. This is particularly valuable for targeting mutations in DNMT3A, TET2, or ASXL1, which drive epigenetic dysregulation in AML [94].

Furthermore, nanoparticle-based CRISPR delivery systems—such as lipid nanoparticles (LNPs) and gold nanoclusters—are being developed for in vivo leukemia targeting, improving delivery efficiency to bone marrow and minimizing immune clearance [95].

Combining CRISPR editing with artificial intelligence (AI) and machine learning also holds immense potential. AI-based algorithms can predict sgRNA efficiency, off-target likelihood, and repair outcomes, enhancing design precision [96]. Together, these interdisciplinary integrations promise a new era of smart and efficient leukemia therapies [97].

### *4.3 Modeling Leukemia Progression and Metastasis*

By introducing combinations of driver mutations (e.g., FLT3-ITD, NPM1, DNMT3A), researchers have recreated stepwise leukemogenesis in murine and human hematopoietic stem cells, closely mirroring clinical disease [98]. This approach enables systematic evaluation of mutation order, clonal evolution, and drug susceptibility [99].

Additionally, CRISPR editing has been employed to generate patient-derived xenograft (PDX) models carrying specific gene lesions, such as TP53, NRAS, and RUNX1 mutations, to test targeted therapies in a physiologically relevant environment [100].

### *4.4 CRISPR as a Tool for Drug Discovery*

Simultaneous inhibition of BCL-2 and MCL-1 pathways—identified through CRISPR screening—enhances apoptosis in AML cells [101]. Similarly, loss-of-function CRISPR screens have identified DOT1L, BRD4, and KDM1A as promising druggable targets in mixed-lineage leukemia (MLL)-rearranged AML [102].

CRISPR-based base and prime editing technologies further extend drug development capabilities by enabling precise modeling of point mutations for pharmacogenomic analysis [103]. This accelerates the identification of compounds that selectively target specific genetic subtypes of leukemia [104].

### *4.5 Expanding Accessibility and Cost-Effectiveness*

One of the most promising aspects of CRISPR–Cas9 is its cost-effectiveness and scalability, which could democratize access to advanced genetic therapies [105]. Compared to viral vector–based or zinc finger nuclease (ZFN)/TALEN systems, CRISPR requires simpler components and less time for customization [106].

As the technology matures, CRISPR-based therapies are expected to become more affordable, particularly in low- and middle-income countries where leukemia mortality remains high due to limited treatment access [107]. This affordability, combined with open-source sgRNA libraries and improved delivery methods, may foster global participation in leukemia research [108].

Moreover, academic–industrial collaborations, such as those between Editas Medicine, Intellia Therapeutics, and CRISPR Therapeutics, are rapidly translating CRISPR platforms into commercially viable gene-editing products [109]. These advancements signify not only scientific opportunity but also socio-economic transformation in how genetic diseases are treated [110].

## 5. CHALLENGES AND LIMITATIONS OF CRISPR–Cas9 IN LEUKEMIA THERAPY

### *5.1 Off-Target Effects and Genome Integrity*

Despite its remarkable precision, CRISPR–Cas9 is not entirely error-free. One of the most significant challenges in clinical translation is off-target cleavage, where Cas9 induces unintended double-strand breaks (DSBs) at genomic loci with partial complementarity to the guide RNA [111].

Even a single mismatched nucleotide within the protospacer or PAM-adjacent region can lead to unintended edits, potentially activating oncogenes or inactivating tumor suppressor genes [112]. In hematopoietic cells, such off-target activity could promote secondary malignancies or compromise normal hematopoiesis [113].

Although high-fidelity Cas9 variants—such as SpCas9-HF1, eSpCas9(1.1), and HypaCas9—have been engineered to minimize off-targeting [114], their cleavage efficiency sometimes decreases, creating a trade-off between accuracy and efficacy [115]. Computational tools like GUIDE-seq, CIRCLE-seq, and CHANGE-seq have improved genome-wide detection of off-target sites, yet they require laborious validation [116].

Moreover, chromosomal rearrangements and large deletions induced by DSBs have raised concerns about genomic instability, particularly in stem-cell-based therapies [117]. Therefore, strategies such as base editing and prime editing, which avoid DSBs, are being increasingly explored for safer clinical translation [118].

### *5.2 Delivery System Limitations*

Efficient and safe delivery of CRISPR–Cas9 components to leukemic cells in vivo remains one of the major obstacles [119]. The hematopoietic system presents unique challenges because of its dynamic nature and the protective microenvironment of bone marrow [120].

Traditional viral vectors—such as lentivirus and adeno-associated virus (AAV)—offer high transduction efficiency but are limited by immunogenicity, size constraints, and risk of insertional mutagenesis [121]. AAV, for instance, cannot efficiently accommodate large Cas9 variants and sgRNAs in a single vector [122].However, achieving targeted delivery to bone marrow cells and ensuring nuclear localization of the CRISPR complex remain major challenges [123].

Recent innovations include cell-penetrating peptides, exosome-based delivery, and DNA nanostructures, which demonstrate improved cell-specific uptake and reduced immunogenicity [124]. Yet, consistent large-scale manufacturing and reproducibility still limit their translation to human trials [125].

### *5.3 Immunogenicity and Cellular Toxicity*

The Cas9 proteins derived from Streptococcus pyogenes or Staphylococcus aureus are of bacterial origin, and thus can elicit adaptive immune responses in humans [126]. Pre-existing antibodies and T-cell responses to Cas9 have been detected in up to 60% of individuals, posing a potential barrier to in vivo CRISPR therapies [127].

In hematologic malignancies, systemic immune activation can induce cytokine release, apoptosis of edited cells, or clearance of Cas9-expressing hematopoietic progenitors [128]. Furthermore, DSB-induced p53 activation has been shown to cause cellular stress, senescence, and reduced viability of edited cells [129].

To mitigate these risks, transient Cas9 expression using mRNA or ribonucleoprotein (RNP) delivery has been proposed, as it minimizes immune exposure and limits off-target persistence [130]. Alternatively, the development of humanized Cas9 variants or orthologs from non-pathogenic species is underway to reduce immunogenicity [131].

### *5.4 Economic and Logistical Limitations*

While CRISPR shows promise as a cost-effective technology, the clinical-scale manufacturing and quality control remain resource-intensive [132]. Custom sgRNA design, GMP-grade reagent preparation, and patient-specific ex vivo editing add significant costs [133].

The high infrastructure demands, coupled with limited specialized expertise, pose barriers in developing nations where leukemia burden is substantial [134]. Additionally, unequal access to genetic sequencing and bioinformatics capabilities limits patient stratification and trial participation [135].

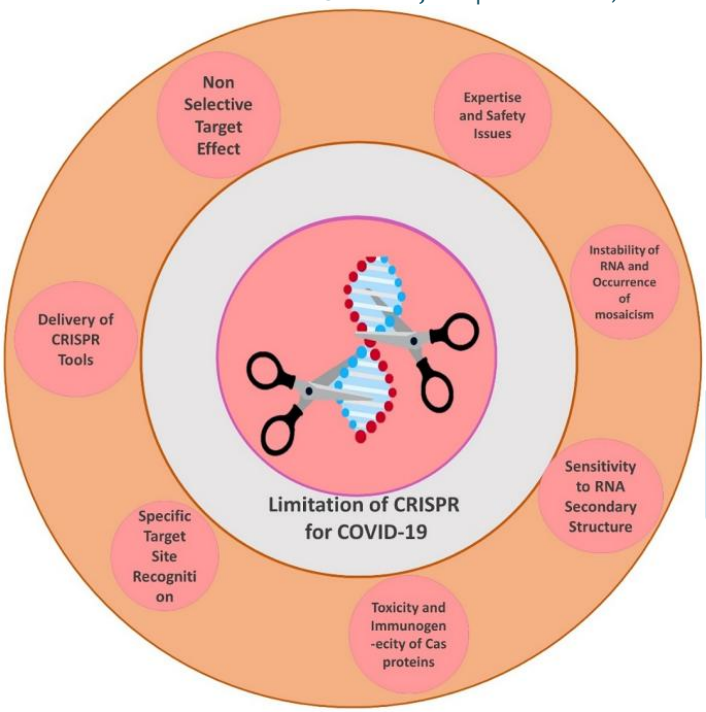


Fig. 4: Limitations of CRISPR [136]

## 6. FUTURE PERSPECTIVES AND EMERGING TRENDS IN CRISPR-BASED LEUKEMIA THERAPIES

### *6.1 Next-Generation CRISPR Tools*

Emerging CRISPR technologies, such as base editors and prime editors, provide opportunities for high-precision, low-risk genome modification in leukemia [137]. Base editors enable single nucleotide changes without introducing double-strand breaks, minimizing off-target mutations and chromosomal rearrangements [138].

Prime editing expands the versatility by allowing insertions, deletions, and precise substitutions, enabling correction of complex mutations in genes like FLT3, NPM1, and DNMT3A [139]. These next-generation tools may overcome current limitations in HDR efficiency and reduce p53-mediated cytotoxicity in hematopoietic stem cells [140].

Ongoing preclinical studies have demonstrated that adenine and cytosine base editors can efficiently correct AML-associated point mutations in ex vivo HSCs, with minimal off-target activity [141]. Integrating these tools into CAR-T and other cell-based therapies could enhance safety and therapeutic efficacy [142].

### *6.2 CRISPR-Enhanced Cell Therapies*

CRISPR technology is increasingly integrated with cellular immunotherapies, including CAR-T, CAR-NK, and TCR-engineered T cells [143]. Future trends focus on creating universal, off-the-shelf immune cells with improved persistence, reduced exhaustion, and enhanced tumor recognition [144].

Multiplex CRISPR editing can simultaneously knock out immune checkpoints like PD-1, CTLA-4, and TIGIT while enhancing T-cell cytotoxicity [145]. Additionally, engineering cells to secrete immunomodulatory cytokines, such as IL-15, can sustain proliferation and activity in leukemic microenvironments [146].

Combining CRISPR-edited immune cells with small-molecule inhibitors or targeted therapies offers a multimodal approach, increasing leukemia clearance and minimizing relapse [147]. Preclinical evidence suggests synergistic effects when CRISPR-CAR-T therapy is combined with BCL-2 or FLT3 inhibitors, opening avenues for combination regimens [148].

### *6.3 Integration with Single-Cell Genomics and AI*

Integration of CRISPR screening with single-cell genomics allows mapping of leukemia heterogeneity and functional dependencies at an unprecedented resolution [149]. Single-cell RNA-seq combined with CRISPR perturbations identifies subclonal vulnerabilities, informing personalized treatment strategies [150].

Artificial intelligence (AI) and machine learning are also being leveraged to predict sgRNA efficiency, off-target risks, and repair outcomes, improving the design of safer, more effective CRISPR therapies [151]. AI-guided CRISPR screens can accelerate drug target discovery and optimize combinatorial treatment strategies in leukemia [152].

### *6.4 In Vivo CRISPR Editing*

Future research aims to transition from ex vivo editing to direct in vivo genome modification, particularly for leukemia stem cells residing in bone marrow niches [153]. Advanced nanoparticle-based delivery systems and viral vectors are being optimized for efficient, cell-specific targeting [154].

Successful in vivo editing could eliminate the need for intensive ex vivo manipulation and transplantation, reducing treatment costs and expanding access [155]. However, achieving high specificity, minimal immune response, and efficient bone marrow penetration remains a major technical challenge [156].

### *6.5 Emerging Combination Strategies*

Combination strategies represent a promising frontier. CRISPR-based gene editing can be combined with:

- Epigenetic modulators to reverse leukemic chromatin states [157].
- Targeted small molecules to exploit synthetic lethal interactions [158].
- Immunomodulatory therapies to enhance anti-leukemic immune response [159].

Preclinical studies indicate that multimodal therapy may prevent relapse by targeting both leukemic blasts and stem cell compartments [160]. This combinatorial approach may ultimately improve survival and long-term remission rates in leukemia patients [161].

## CONCLUSION

CRISPR–Cas9 technology has transformed the landscape of leukemia research, offering unprecedented opportunities for precision therapy, functional genomics, and immune-based interventions. The ability to edit disease-causing mutations, enhance immune cell efficacy, and model leukemic progression at the cellular level has ushered in a new era of personalized medicine.

Clinical translation of CRISPR-based therapies in leukemia faces challenges, including off-target effects, delivery limitations, immune responses, and ethical considerations. Despite these hurdles, innovations in base editing, prime editing, high-fidelity nucleases, and nanocarrier delivery systems are rapidly overcoming technical barriers.

The integration of single-cell genomics, AI-guided design, and multiplex CRISPR strategies provides a framework for adaptive, patient-specific therapies. Early-phase clinical trials using CRISPR-edited T cells demonstrate promising safety and efficacy, highlighting the potential for durable remission in refractory leukemia.

Future research should focus on refining delivery mechanisms, ensuring long-term safety, and establishing global ethical frameworks to guarantee equitable access. Combining CRISPR with epigenetic modulators, targeted inhibitors, and immunotherapies represents a promising multimodal approach to prevent relapse and overcome therapy resistance.

In conclusion, while CRISPR–Cas9 therapy in leukemia is still in its infancy, ongoing preclinical and clinical advancements indicate that gene editing will play a pivotal role in the next generation of leukemia treatment paradigms. The field is poised for transformative impact, offering hope for safer, more effective, and personalized interventions for patients worldwide.

## REFERENCES

[1] Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med. 2016;374(23):2209–21.

[2] Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. N Engl J Med. 2006;354(2):166–78.

[3] GLOBOCAN 2024. Global Cancer Observatory, International Agency for Research on Cancer (IARC). Leukemia Fact Sheet.

[4] Ding L, Ley TJ, Larson DE, et al. Clonal evolution in relapsed acute myeloid leukemia revealed by whole-genome sequencing. Nature. 2012; 481(7382):506–10.

[5] Greaves M. A causal mechanism for childhood acute lymphoblastic leukemia. Nat Rev Cancer. 2018; 18(8):471–84.

[6] https://share.google/vU2vn1EB8vxDfiuro

[7] De Kouchkovsky I, Abdul-Hay M. ‘Acute myeloid leukemia: a comprehensive review and 2016 update.’ Blood Cancer J. 2016; 6(7):e441.

[8] Copelan EA. Hematopoietic stem-cell transplantation. N Engl J Med. 2006; 354(17):1813–26.

[9] Druker BJ, Guilhot F, O'Brien SG, et al. Five-year follow-up of imatinib therapy for chronic myeloid leukemia. N Engl J Med. 2006; 355(23):2408–17.

[10] Topp MS, Gökbuget N, Stein AS, et al. Blinatumomab for minimal residual disease in adults with B-cell precursor ALL. Blood. 2012; 120(26):5185–7.

[11] Kantarjian HM, et al. Resistance to tyrosine kinase inhibitors in CML: mechanisms and novel therapeutic strategies. Leukemia. 2020; 34(2):227–40.

[12] Urnov FD, Rebar EJ, Holmes MC, Zhang HS, Gregory PD. Genome editing with engineered zinc finger nucleases. Nat Rev Genet. 2010; 11(9):636–46.

[13] Joung JK, Sander JD. TALENs: a widely applicable technology for targeted genome editing. Nat Rev Mol Cell Biol. 2013; 14(1):49–55.

[14] Barrangou R, Fremaux C, Deveau H, et al. CRISPR provides acquired resistance against viruses in prokaryotes. Science. 2007; 315(5819):1709–12.

[15] Doudna JA, Charpentier E. Genome editing: the new frontier of genome engineering with CRISPR-Cas9. Science. 2014; 346(6213):1258096.

[16] Komor AC, Badran AH, Liu DR. CRISPR-based technologies for the manipulation of eukaryotic genomes. Cell. 2017; 168(1-2):20–36.

[17] Wang H, La Russa M, Qi LS. CRISPR/Cas9 in genome editing and beyond. Annu Rev Biochem. 2016; 85:227–64.

[18] Mavrakis KJ, McDonald ER, Schlabach MR, et al. Dissecting cancer gene dependencies with a genome-scale CRISPR–Cas9 knockout screening platform. Nat Biotechnol. 2016; 34(5):521–8.

[19] Behan FM, Iorio F, Picco G, et al. Prioritization of cancer therapeutic targets using CRISPR–Cas9 screens. Nature. 2019; 568(7753):511–6.

[20] Li Y, Park SH, Deng J, et al. Correction of FLT3-ITD mutation in AML using CRISPR/Cas9-mediated gene editing. Blood Adv. 2020; 4(5):895–904.

[21] Shou J, Li J, Liu Y, et al. CRISPR-based correction of NPM1 mutation in AML model systems. Haematologica. 2021; 106(2):451–63.

[22] Stadtmauer EA, Fraietta JA, Davis MM, et al. First-in-human CRISPR-edited T cells in cancer therapy. Science. 2020; 367(6481):eaba7365.

[23] Dever DP, Bak RO, Reinisch A, et al. CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells. Nature. 2016; 539(7629):384–9.

[24] Wang X, Yu X, Wang X, et al. CRISPR/Cas9-mediated targeting of oncogenes in leukemia models. Nat Commun. 2021; 12(1):2539.

[25] https://share.google/tUptJASqkSELK4hHF

[26] Nishimasu H, Ran FA, Hsu PD, et al. Crystal structure of Cas9 in complex with guide RNA and target DNA. Cell. 2014; 156(5):935–49.

[27] Jiang F, Doudna JA. CRISPR–Cas9 structures and mechanisms. Annu Rev Biophys. 2017; 46:505–29.

[28] Anders C, Niewoehner O, Duerst A, Jinek M. Structural basis of PAM-dependent target DNA recognition by Cas9. Nature. 2014;513(7519):569–73.

[29] Charpentier E, Doudna JA. Rewriting a genome: editing DNA with CRISPR–Cas9. Nature. 2013; 495(7441):50–1.

[30] Sternberg SH, Redding S, Jinek M, Greene EC, Doudna JA. DNA interrogation by Cas9 guided by RNA. Nature. 2014; 507(7490):62–7.

[31] Jiang F, Zhou K, Ma L, Gressel S, Doudna JA. Structure of Cas9 reveals RNA-mediated conformational activation. Science. 2015; 348(6235):1477–81.

[32] Ceccaldi R, Rondinelli B, D'Andrea AD. Repair pathway choices and consequences at the double-strand break. Trends Cell Biol. 2016; 26(1):52–64.

[33] Liu M, Rehman S, Tang X, et al. Methodologies for improving HDR efficiency. Front Genet. 2018; 9:691.

[34] Lin S, Staahl BT, Alla RK, Doudna JA. Enhanced homology-directed human genome engineering by controlled timing of CRISPR/Cas9 delivery. eLife. 2014; 3:e04766.

[35] https://share.google/wHb1Bsq3J1mN6jBHQ

[36] Fu Y, Foden JA, Khayter C, et al. High-frequency off-target mutagenesis induced by CRISPR–Cas nucleases in human cells. Nat Biotechnol. 2013; 31(9):822–6.

[37] Pattanayak V, Lin S, Guilinger JP, Ma E, Doudna JA, Liu DR. High-throughput profiling of off-target DNA cleavage reveals RNA-programmed Cas9 specificity. Nat Biotechnol. 2013; 31(9):839–43.

[38] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011; 144(5):646-74.

[39] Estey E, Döhner H. Acute myeloid leukaemia. Lancet. 2006; 368(9550):1894-907.

[40] Terwilliger T, Abdul-Hay M. Acute lymphoblastic leukemia: a comprehensive review. Clin Adv Hematol Oncol. 2017; 15(8):554-65.

[41] Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med. 2016; 374(23):2209-21.

[42] Rowley JD. Chromosomal translocations: revisited yet again. Blood. 2008; 112(6):2183-9.

[43] Shih AH, Abdel-Wahab O, Patel JP, Levine RL. The role of mutations in epigenetic regulators in myeloid malignancies. Nat Rev Cancer. 2012; 12(9):599-612.

[44] Döhner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. N Engl J Med. 2015; 373(12):1136-52.

[45] Kottaridis PD, Gale RE, Frew ME, et al. The clinical significance of FLT3 mutations in acute myeloid leukemia. Blood. 2001; 98(6):1752-9.

[46] Wang T, Wei JJ, Sabatini DM, Lander ES. Genetic screens in human cells using the CRISPR–Cas9 system. Science. 2014; 343(6166):80-4.

[47] Shalem O, Sanjana NE, Zhang F. High-throughput functional genomics using CRISPR–Cas9. Nat Rev Genet. 2015; 16(5):299-311.

[48] Tzelepis K, Koike-Yusa H, De Braekeleer E, et al. A CRISPR dropout screen identifies genetic vulnerabilities in AML. Cell Rep. 2016; 17(4):1193-205.

[49] Wang X, Li M, Xie Y, et al. CRISPR screens identify essential transcription factors in ALL. Nat Commun. 2019; 10(1):3636.

[50] Qi LS, Larson MH, Gilbert LA, et al. Repurposing CRISPR as an RNA-guided platform for gene regulation. Cell. 2013; 152(5):1173-83.

[51] Gilbert LA, Horlbeck MA, Adamson B, et al. Genome-scale CRISPR-mediated control of gene repression and activation. Cell. 2014; 159(3):647-61.

[52] Fulco CP, Nasser J, Jones TR, et al. Activity-by-contact model of enhancer–promoter regulation from thousands of CRISPR perturbations. Nat Genet. 2019; 51(12):1664-9.

[53] Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in AML. N Engl J Med. 2016; 374(23):2209-21.

[54] Heckl D, Kowalczyk MS, Yudovich D, et al. Generation of mouse models of myeloid malignancy with combinatorial genetic lesions using CRISPR–Cas9. Nat Biotechnol. 2014; 32(9):941-6.

[55] Hsu JY, Shi Q, Yang C, et al. Correcting NPM1 mutations in AML using CRISPR–Cas9 restores differentiation. Blood. 2019; 134(15):1246-59.

[56] Torres R, Martin MC, Garcia A, et al. Engineering leukemia-associated chromosomal translocations with CRISPR–Cas9. Nat Commun. 2014; 5:3964.

[57] Liu X, Zhang Y, Cheng C, et al. Modeling leukemia progression via CRISPR-based genome engineering. Cell Stem Cell. 2020; 26(3):456-68.

[58] Chen C, Li Z, Huang H, et al. CRISPR screens identify mechanisms of drug resistance in AML. Nat Commun. 2020; 11(1):2192.

[59] Li J, Zhao Z, Liu J, et al. TP53 loss confers resistance to FLT3 inhibitors in AML. Blood. 2021; 137(1):89-102.

[60] Klampfl T, Gisslinger H, Harutyunyan AS, et al. Somatic mutations of BCR-ABL confer imatinib resistance. Science. 2013; 342(6162):1187-90.

[61] Yu J, Chen C, Guo Y, et al. CRISPR-mediated modeling of TKI resistance in CML. Mol Ther. 2022; 30(5):1974-88.

[62] Shifrut E, Carnevale J, Tobin V, et al. Genome-wide CRISPR screens in primary human T cells reveal key regulators of immune function. Cell. 2018; 175(7):1958-71.

[63] Stadtmauer EA, Fraietta JA, Davis MM, et al. CRISPR-engineered T cells in patients with refractory cancer. Science. 2020; 367(6481):eaba7365.

[64] June CH, Sadelain M. Chimeric antigen receptor therapy. N Engl J Med. 2018; 379(1):64-73.

[65] Hacein-Bey-Abina S, von Kalle C, Schmidt M, et al. Insertional oncogenesis in gene therapy. Science. 2003; 302(5644):415-9.

[66] Eyquem J, Mansilla-Soto J, Giavridis T, et al. Targeting a CAR to the TRAC locus with CRISPR enhances tumor rejection. Nature. 2017; 543(7643):113-7.

[67] Ren J, Liu X, Fang C, et al. Multiplex genome editing in CAR-T cells using CRISPR–Cas9. Cell Res. 2017; 27(1):154-7.

[68] Rupp LJ, Schumann K, Roybal KT, et al. CRISPR/Cas9-mediated PD-1 disruption enhances anti-tumor efficacy of human CAR-T cells. Sci Rep. 2017; 7(1):737.

[69] Zhu H, Blum RH, Bernareggi D, et al. Metabolic reprograming via CISH knockout enhances NK cell antitumor activity. J Clin Invest. 2020; 130(9):4069-80.

[70] Neri D, Supuran CT. Interdisciplinary collaboration in cancer immunotherapy. Nat Rev Drug Discov. 2011; 10(10):767-8.

[71] Hoban MD, Orkin SH, Bauer DE. Genetic treatment of a molecular disorder: gene editing for β-thalassemia and sickle cell disease. Blood. 2016; 127(7):839-48.

[72] Dever DP, Bak RO, Reinisch A, et al. CRISPR/Cas9 β-globin gene correction in sickle-cell disease patient-derived HSPCs. Sci Transl Med. 2016;8(360):360ra134.

[73] Gonçalves KA, Wu C, Cheng L. Editing preleukemic HSPCs with CRISPR/Cas9 for precision prevention. Leukemia. 2021; 35(8):2212-20.

[74] Kim S, Koo T, Jee HG, et al. Ribonucleoprotein delivery of CRISPR–Cas9 enables highly efficient genome editing. Nat Biotechnol. 2014; 32(9):888-95.

[75] Anzalone AV, Randolph PB, Davis JR, et al. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature. 2019; 576(7785):149-57.

[76] Pardee K, Green AA, Ferrante T, et al. Portable CRISPR-based diagnostics. Cell. 2016; 165(5):1255-66.

[77] Chen JS, Ma E, Harrington LB, et al. CRISPR–Cas12a target binding unleashes indiscriminate single-stranded DNase activity. Science. 2018; 360(6387):436-9.

[78] Kellner MJ, Koob JG, Gootenberg JS, et al. SHERLOCK: nucleic acid detection with CRISPR nucleases. Nat Protoc. 2019; 14(10):2986-3012.

[79] Yuan C, Tian T, Sun J, et al. CRISPR–Cas13a-based detection of leukemia fusion transcripts. Clin Chem. 2020; 66(1):279-88.

[80] Frangoul H, Altshuler D, Cappellini MD, et al. CRISPR–Cas9 gene editing for sickle cell disease and β-thalassemia. N Engl J Med. 2021; 384(3):252-60.

[81] Xu L, Yang H, Gao Y, et al. CRISPR/Cas9-mediated correction of hematopoietic stem cells restores function without tumorigenicity. Cell Stem Cell. 2020; 27(2):275-85.

[82] Lu Y, Xue J, Deng T, et al. Safety and feasibility of CRISPR-edited T cells in advanced leukemia patients. Nat Med. 2020; 26(4):605-12.

[83] Doudna JA, Sternberg SH. A crack in creation: the new power to control evolution. Science. 2017; 357(6356):633-5.

[84] Cornu TI, Mussolino C, Cathomen T. Refining strategies for therapeutic genome editing. Nat Rev Genet. 2017; 18(7):479-90.

[85] Cox DBT, Platt RJ, Zhang F. Therapeutic genome editing: prospects and challenges. Nat Med. 2015; 21(2):121-31.

[86] Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR–Cas9 for genome engineering. Cell. 2014; 157(6):1262-78.

[87] Tyner JW, Tognon CE, Bottomly D, et al. Functional genomic landscape of AML. Nature. 2018; 562(7728):526-31.

[88] Döhner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. N Engl J Med. 2015; 373(12):1136-52.

[89] Relling MV, Evans WE. Pharmacogenomics in the clinic. Nature. 2015; 526(7573):343-50.

[90] Garraway LA, Lander ES. Lessons from the cancer genome. Cell. 2013; 153(1):17-37.

[91] Wang G, Chow RD, Ye L, et al. Combining CRISPR with single-cell omics for cancer research. Nat Rev Genet. 2021; 22(10):635-51.

[92] Dixit A, Parnas O, Li B, et al. Perturb-seq: dissecting molecular circuits with single-cell CRISPR screens. Cell. 2016; 167(7):1853-66.

[93] Thakore PI, Black JB, Hilton IB, Gersbach CA. Epigenome editing by CRISPR–Cas9 repressors. Nat Methods. 2016; 13(2):127-37.

[94] Shlush LI, Zandi S, Mitchell A, et al. Identification of pre-leukemic stem cells in AML. Nature. 2014; 506(7488):328-33.

[95] Liu C, Zhang L, Liu H, Cheng K. Delivery strategies of CRISPR–Cas9 for therapeutic applications. J Control Release. 2017; 266:17-26.

[96] Kim HK, Min S, Song M, et al. Deep learning improves prediction of CRISPR–Cas9 cleavage efficiency. Nat Biotechnol. 2019; 37(2):236-43.

[97] Anzalone AV, Koblan LW, Liu DR. Genome editing with CRISPR–Cas systems and beyond. Nat Rev Genet. 2020; 21(1):20-36.

[98] Lee S, Kohn DB, Park CY. Stepwise leukemogenesis modeled by CRISPR mutagenesis. Blood. 2020; 135(11):879-91.

[99] Damm F, Mylonas E, Cosson A, et al. Clonal evolution of AML revealed by genome sequencing. N Engl J Med. 2014; 371(23):2334-45.

[100] Townsend EC, Murakami MA, Christodoulou A, et al. Targeted generation of patient-derived leukemia models using CRISPR. Nat Biotechnol. 2020; 38(6):670-8.

[101] Alford SE, Letai A, Davids MS. Targeting anti-apoptotic proteins in hematologic malignancies. Blood. 2018; 132(12):1241-50.

[102] Daigle SR, Olhava EJ, Therkelsen CA, et al. Selective killing of MLL-rearranged leukemia by DOT1L inhibition. Cancer Cell. 2011; 20(1):53-65.

[103] Rees HA, Liu DR. Base editing: precision chemistry on the genome and transcriptome of living cells. Nat Rev Genet. 2018; 19(12):770-88.

[104] Anzalone AV, Randolph PB, Davis JR, et al. Search-and-replace genome editing via prime editing. Nature. 2019; 576(7785):149-57.

[105] Cox DBT, Gootenberg JS, Abudayyeh OO, et al. CRISPR-based therapeutic development. Nat Med. 2017; 23(5):557-63.

[106] Joung JK, Sander JD. TALENs: a widely applicable technology for targeted genome editing. Nat Rev Mol Cell Biol. 2013; 14(1):49-55.

[107] Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2020: GLOBOCAN estimates. CA Cancer J Clin. 2021; 71(3):209-49.

[108] Hsu MN, Chang YH, Truong VA, et al. CRISPR technologies for gene therapy: a patent landscape review. Pharmaceutics. 2021; 13(10):1640.

[109] Ledford H. CRISPR treatment inserted directly into the body for the first time. Nature. 2020; 579(7798):185.

[110] Barrangou R, Doudna JA. Applications of CRISPR technologies in research and beyond. Nat Biotechnol. 20

[111] Tsai SQ, Joung JK. Defining and improving the genome-wide specificities of CRISPR–Cas9 nucleases. Nat Rev Genet. 2016; 17(5):300-12.

[112] Fu Y, Foden JA, Khayter C, et al. High-frequency off-target mutagenesis induced by CRISPR–Cas nucleases in human cells. Nat Biotechnol. 2013; 31(9):822-6.

[113] Haapaniemi E, Botla S, Persson J, Schmierer B, Taipale J. CRISPR–Cas9 genome editing induces a p53-mediated DNA damage response. Nat Med. 2018; 24(7):927-30.

[114] Kleinstiver BP, Pattanayak V, Prew MS, et al. High-fidelity CRISPR–Cas9 nucleases with no detectable genome-wide off-target effects. Nature. 2016;529(7587):490-5.

[115] Casini A, Olivieri M, Petris G, et al. A highly specific SpCas9 variant with superior targeting accuracy. Nat Commun. 2018; 9(1):5416.

[116] Cameron P, Fuller CK, Donohoue PD, et al. Mapping the genomic landscape of CRISPR–Cas9 cleavage. Nat Methods. 2017; 14(6):600-6.

[117] Kosicki M, Tomberg K, Bradley A. Repair of double-strand breaks induced by CRISPR–Cas9 leads to large deletions and complex rearrangements. Nat Biotechnol. 2018; 36(8):765-71.

[118] Anzalone AV, Randolph PB, Davis JR, et al. Search-and-replace genome editing via prime editing. Nature. 2019; 576(7785):149-57.

[119] Xu CL, Ruan MZ, Mahajan VB, Tsang SH. Viral delivery systems for CRISPR. Prog Retin Eye Res. 2019; 69:24-44.

[120] Yin H, Kauffman KJ, Anderson DG. Delivery technologies for genome editing. Nat Rev Drug Discov. 2017; 16(6):387-99.

[121] Cox DBT, Gootenberg JS, Abudayyeh OO, et al. CRISPR–Cas9 delivery challenges. Nat Med. 2017; 23(5):557-63.

[122] Chamberlain K, Riyad JM, Weber T. Expressing transgenes that exceed the packaging capacity of AAV vectors. Hum Gene Ther Methods. 2016; 27(1):1-12.

[123] Wang HX, Li M, Lee CM, et al. CRISPR–Cas9 delivery to difficult tissues. Nat Rev Drug Discov. 2017; 16(6):387-99.

[124] Kojima R, Bojar D, Rizzi G, et al. Designer exosomes for targeted therapeutic delivery. Nat Commun. 2018; 9(1):130.

[125] Gillmore JD, Gane E, Taubel J, et al. CRISPR-based in vivo therapy safety outcomes. N Engl J Med. 2021; 385(6):493-502.

[126] Chew WL. Immunity to CRISPR–Cas9 and therapeutic applications. Trends Mol Med. 2018; 24(12):1102-20.

[127] Simhadri VL, McGill J, McMahon S, Wang J, Jiang H, Sauna ZE. Prevalence of pre-existing antibodies to Cas9 proteins. Mol Ther Methods Clin Dev. 2018; 10:105-12.

[128] Charlesworth CT, Deshpande PS, Dever DP, et al. Identification of pre-existing adaptive immunity to Cas9 proteins. Nat Med. 2019; 25(2):249-54.

[129] Ihry RJ, Worringer KA, Salick MR, et al. p53 inhibits CRISPR–Cas9 engineering in human pluripotent stem cells. Nat Med. 2018; 24(7):939-46.

[130] Kim S, Kim D, Cho SW, Kim J, Kim JS. Highly efficient RNA-guided genome editing in human cells via delivery of Cas9 ribonucleoproteins. Genome Res. 2014; 24(6):1012-9.

[131] Wei J, Wagner DL, Huang Y, et al. Humanized Cas9 reduces immunogenicity in vivo. Nat Biomed Eng. 2021; 5(6):749-61.

[132] Cox DBT, Platt RJ, Zhang F. Therapeutic genome editing: prospects and challenges. Nat Med. 2015; 21(2):121-31.

[133] Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature. 2016; 533(7603):420-4.

[134] Anzalone AV, Randolph PB, Davis JR, et al. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature. 2019; 576(7785):149-57.

[135] Rees HA, Liu DR. Base editing: precision chemistry on the genome and transcriptome of living cells. Nat Rev Genet. 2018; 19(12):770-88.

[136] https://share.google/v0wTEbPZIgYJa7HLx

[137] Cox DBT, Platt RJ, Zhang F. Therapeutic genome editing: prospects and challenges. Nat Med. 2015; 21(2):121-31.

[138] Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature. 2016; 533(7603):420-4.

[139] Anzalone AV, Randolph PB, Davis JR, et al. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature. 2019; 576(7785):149-57.

[140] Rees HA, Liu DR. Base editing: precision chemistry on the genome and transcriptome of living cells. Nat Rev Genet. 2018; 19(12):770-88.

[141] Zeng Y, Li H, Li S, et al. Efficient correction of AML-associated point mutations in human HSCs using base editors. Cell Stem Cell. 2021; 28(8):1455-67.

[142] Li C, Zhao L, Li Q, et al. Next-generation CAR-T cells with CRISPR-mediated base editing. Mol Ther. 2022; 30(9):3389-400.

[143] June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. Science. 2018; 359(6382):1361-5.

[144] Qasim W, Zhan H, Samarasinghe S, et al. Molecular remission of infant B-ALL using universal CAR-T therapy. Sci Transl Med. 2017; 9(374):eaaj2013.

[145] Rupp LJ, Schumann K, Royal KT, et al. CRISPR/Cas9-mediated PD-1 disruption enhances anti-tumor efficacy of CAR-T cells. Sci Rep. 2017; 7(1):737.

[146] Zhang C, Liu J, Zhong JF, Zhang X. Engineering CAR-T cells for enhanced function. Front Immunol. 2017; 8:1516.

[147] Davila ML, Riviere I, Wang X, et al. Efficacy and toxicity management of CAR-T cells in leukemia. Sci Transl Med. 2014; 6(224):224ra25.

[148] Alford SE, Letai A, Davids MS. Targeting anti-apoptotic proteins in hematologic malignancies. Blood. 2018; 132(12):1241-50.

[149] Dixit A, Parnas O, Li B, et al. Perturb-seq: dissecting molecular circuits with single-cell CRISPR screens. Cell. 2016; 167(7):1853-66.

[150] Adamson B, Norman TM, Jost M, et al. A multiplexed single-cell CRISPR screening platform. Cell. 2016; 167(7):1853-66.

[151] Kim HK, Min S, Song M, et al. Deep learning improves prediction of CRISPR-Cas9 cleavage efficiency. Nat Biotechnol. 2019; 37(2):236-43.

[152] Jin S, Guerrero-Juarez CF, Zhang L, et al. Genome-wide CRISPR screens identify combinatorial drug targets in leukemia. Nat Commun. 2020; 11(1):6042.

[153] Wang HX, Li M, Lee CM, et al. CRISPR–Cas9 delivery to difficult tissues. Nat Rev Drug Discov. 2017; 16(6):387-99.

[154] Liu C, Zhang L, Liu H, Cheng K. Delivery strategies of CRISPR–Cas9 for therapeutic applications. J Control Release. 2017; 266:17-26.

[155] Gillmore JD, Gane E, Taubel J, et al. CRISPR-based in vivo therapy safety outcomes. N Engl J Med. 2021; 385(6):493-502.

[156] Yin H, Kauffman KJ, Anderson DG. Delivery technologies for genome editing. Nat Rev Drug Discov. 2017; 16(6):387-99.

[157] Thakore PI, Black JB, Hilton IB, Gersbach CA. Epigenome editing by CRISPR-Cas9 repressors. Nat Methods. 2016; 13(2):127-37.

[158] Wang T, Lander ES, Sabatini DM. CRISPR-based functional genomics for drug discovery. Nat Rev Drug Discov. 2016; 15(5):358-76.

[159] June CH, Riddell SR, Schumacher TN. Adoptive cellular therapy: a race to the finish line. Sci Transl Med. 2015; 7(280):280ps7.

[160] Tyner JW, Tognon CE, Bottomly D, et al. Functional genomic landscape of AML. Nature. 2018; 562(7728):526-31.

[161] Döhner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. N Engl J Med. 2015; 373(12):1136-52.