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CRITICAL REVIEW

A CRISPR view of hematopoietic stem cells: Moving innovative bioengineering into the clinic

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Abstract

Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas genome engineering has emerged as a powerful tool to modify precise genomic sequences with unparalleled accuracy and efficiency. Major advances in CRISPR technologies over the last 5 years have fueled the development of novel techniques in hematopoiesis research to interrogate the complexities of hematopoietic stem cell (HSC) biology. In particular, high throughput CRISPR based screens using various "flavors" of Cas coupled with sequencing and/or functional outputs are becoming increasingly efficient and accessible. In this review, we discuss recent achievements in CRISPR-mediated genomic engineering and how these new tools have advanced the understanding of HSC heterogeneity and function throughout life. Additionally, we highlight how these techniques can be used to answer previously inaccessible questions and the challenges to implement them. Finally, we focus on their translational potential to both model and treat hematological diseases in the clinic.

1 | INTRODUCTION

Clustered regularly interspaced short palindromic repeats (CRISPR) were first described in *Escherichia coli* in 1987[1] and later found to function with CRISPR associated (Cas) genes as the bacteria's adaptive immunity against phages.[2–6] Since the pioneering work of Dr. Doudna's and Dr. Charpentier's laboratories in 2012[7] that defined the parameters to use programmable CRISPR/Cas9 systems and the subsequent adaptation into eukaryotic cells from Drs. Feng Zhang and George Church's groups,[8,9] CRISPR/Cas systems have revolutionized both basic and clinical science.[10,11] As a powerful tool in the genomic editing toolbox, scientists can not only identify disease causing mutations, but also can correct these mutations to cure disease using CRISPR-based gene therapies. Over 3000 genes with single, disease-causing mutations have been identified,[12] and to date, more than 170 of these genes have been associated with hematopoiesis, highlighting the immense need and potential to design safe and effective CRISPR-based therapeutics. Here, we discuss how innovations in CRISPR/Cas genetic manipulation has advanced basic hematopoietic research over the last decade and how CRISPR-based hematopoietic stem cell (HSC) bioengineering is accelerating clinical approaches to treating hematopoietic disorders.

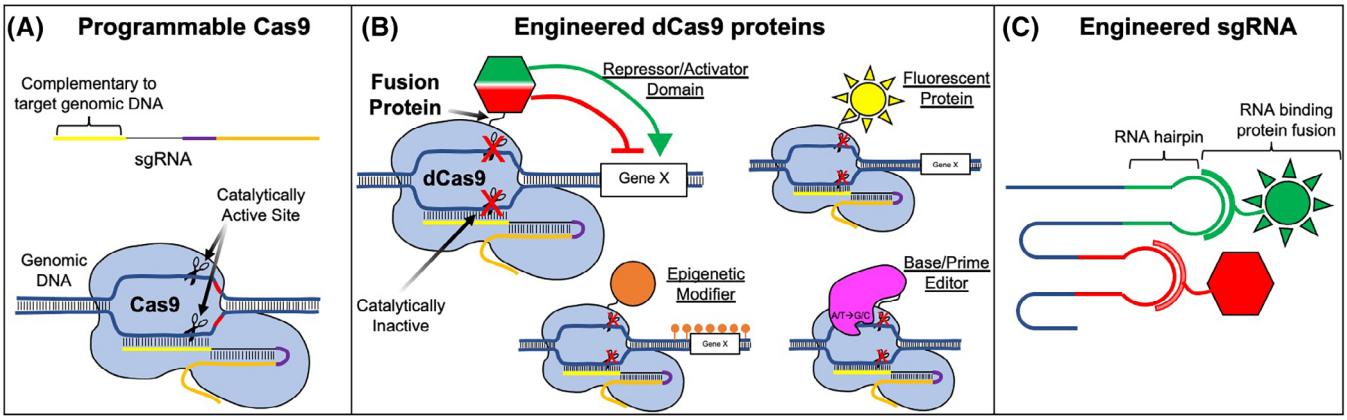

FIGURE 1 Engineered CRISPR/Cas9 systems. (A) The Cas9's endonuclease domains (scissors) induce double-strand DNA breaks in a sequence specific manner that is determined by the complementary sequence of the sgRNA. The guide region (yellow) is ~20 nucleotide long and must be complementary to a DNA sequence next to a PAM sequence, highlighted in red. (B) dCas9 fused to a fluorescent protein allows for intracellular live labeling of genes; a repressor domain like KRAB causes gene silencing; an activator domain like VP64 induces gene expression; epigenetic modifiers such as GCN5 lays down histone acetylation activation marks or Sir2a establishes histone deacetylation repressive marks, or nucleobase deaminase enzymes to randomly swap bases. (C) sgRNA engineered to include RNA hairpins like MS2, PP7 or boxP recruit the RNA binding proteins MCP, PCP and N22, respectively. The RNA binding proteins can be fused to fluorescent proteins or activator/repressor domains. CRISPR, clustered regularly interspaced short palindromic repeats [Color figure can be viewed at wileyonlinelibrary.com]

2 | ENGINEERING CAS PROTEINS AND SYNTHETIC GUIDE RNAs FOR NOVEL RESEARCH PURPOSES

The power of the CRISPR/Cas system is that it can specifically and efficiently target sequences in the genome with just a single synthetic guide RNA (sgRNA) and a single protein (Figure 1A). The sgRNA directs Cas9 to the targeted DNA sequence, where Cas9 creates double stranded breaks, inducing the cell's DNA repair pathways. Non-homologous end joining can result in insertion–deletion (indel) mutations at the target site, while homology-directed repair can incorporate a template DNA, resulting in insertion of new genetic material. Soon after Cas9 was discovered, scientists adapted and modified this system to address a spectrum of research needs. The Cas9 protein has two catalytic domains responsible for creating double-stranded breaks in DNA. A single nucleotide mutation is sufficient to abolish activity of either domain,[7] thus creating the enzymatically inactive, dead-Cas9 (dCas9). Although dCas9 does not cut DNA, it is still capable of binding specific DNA sequences in a guide RNA-dependent manner. By itself, dCas9 can compete for binding with endogenous transcription factors to test their functional roles at specific regulatory elements.[13] When fused to other protein domains, dCas9 can be used to bring select functionalities to genetic loci of interest (Figure 1B). For example, dCas9 fused to fluorescent proteins can be used to visualize the intracellular location of the guide RNA target.[14] dCas9 has also been fused to transcriptional regulatory domains to manipulate gene expression of sgRNA targeted genes.[15,16] For this purpose, scientists have used repressor domains such as KRAB-zinc finger proteins or activators like VP-64. These systems are referred to as CRISPR-interference (CRISPRi) and CRISPR-activation (CRISPRa) that silences or activates gene expression, respectively. Epigenetic modifiers can also be fused to dCas9, and these fusion proteins induce repressive or activating chromatin marks.[17] Furthermore, nucleobase deaminase enzymes can be fused to dCas9 to perform base editing.[18–20] These base editors can convert target nucleotide bases without the need of double-strand breaks. Specifically, cytosine base editors swap cytosine-guanine (C-G) with thymine-adenine (T-A) and adenine base editors swap A-T to G-C.

As an alternative to altering Cas, the sgRNA can be engineered to serve extra purposes. Different types of hairpins introduced into the sgRNA act as scaffolds to recruit additional proteins to specific DNA sequences with dCas9 (Figure 1C). A recently engineered sgRNA includes additional repressor binding domains in the form of an MS2 hairpin, an RNA secondary structure with MS2 coat protein (MCP) binding sites. The MS2 hairpins bind MCPs fused to activators/repressors, creating a dual activator/repressor system if used with CRISPRi/CRISPRa.[21] Other types of hairpins include PP7 that bind PCP and boxP that bind N22. These hairpins can be used in combination to visualize multiple genomic loci when MCP, PCP and N22 are fused to different fluorescent proteins, as in CRISPRainbow.[22]

A recent advance in base editors employ a hybrid model.[23] Such "prime editors" consist of a reverse transcriptase fused to a Cas9 nickase in which one of the two catalytically active site of Cas9 is mutated to be non-functional. Prime editors require special prime-editing guide RNAs that include the template for repair and are capable of correcting all 12 types of point mutations, including insertions and deletions. Each of these flavors of Cas9 and sgRNAs serve as new, powerful tools to pursue novel avenues of research with relative ease and efficiency, compared to other genome editing/manipulation tools (like TALENs and zinc finger nucleases).

3 | CRISPR IN HEMATOPOIESIS

As soon as CRISPR/Cas9 was introduced as a new genome editing tool, its potential for use in the hematopoietic system was highlighted.[9,24] The ease of testing both hematopoietic stem/progenitor and mature cell function via transplantation lends itself to CRISPR-mediated manipulation. As a result, hematopoietic research has significantly advanced with implementation of these technologies. While CRISPR/Cas9 targeting of single genes is an important tool to test gene function in primary hematopoietic cells, a potentially even greater power of CRISPR/Cas9 in hematopoietic research is through high-throughput screens. We highlight some notable advances in CRISPR-based screens using different engineered Cas9 proteins (Figure 1).

3.1 | CRISPR/Cas9 screens

Great progress in understanding HSC biology with regards to HSC function, clonality and hematopoietic hierarchy has been made using single-cell HSC transplants,[25–28] viral barcoding[29–32] and in vivo barcoding.[33–35] Such studies have resolved many questions about HSC differentiation; however, they are unable to answer *how* HSC differentiation potential is regulated. To address this, Amit and colleagues developed CRISP-seq to interrogate the role of known transcription factors in myeloid development and in response to immune stimulation.[36] They coupled pooled CRISPR-mediated genetic perturbations—that is, Cas9 induced gene knockdowns—with single cell RNA sequencing to determine how single or multiple genetic perturbations affect cell identity and function. By targeting the five transcription factors Cebpb, Irf8, Rela, Stat1 and Stat2 simultaneously and in all combinations, they determined that Stat1/2 promoted dendritic cell fate and Cebpb promoted monocytes and macrophages cell fate. Interestingly, upon lipopolysaccharide (LPS)-mediated immune activation, cells with Stat1/2 perturbations downregulated antiviral genes compared to WT cells, demonstrating a role for Stat1/2 in cell function in addition to cell fate. Similarly, Dixit and colleagues developed Perturb-seq to induce site-specific perturbations using CRISPR/Cas9 followed by sequencing to determine how cells transcriptionally respond to those perturbation.[37] It is important to note that these studies rely on single-cell RNA-sequencing which does not as reliably detect more lowly expressed transcripts as bulk RNA-seq; this limitation may be overcome with advances in single cell sequencing technologies. Overall, these experiments showed the power of CRISPR/Cas9 to interrogate multiple functional regulators with single cell resolution.

CRISPR/Cas9-based screens can also be used to understand the function of HSC-derived mature, terminally differentiated cells and enhance different disease treatments. For example, Marson and colleagues used a CRISPR-based screen to understand how the master transcription factor FoxP3 influences regulatory T cell (T_{reg}) function and identify potential targets to enhance T_{reg} antitumor properties.[38] By targeting over 400 nuclear factors in their screen, they identified both positive and negative regulators of FoxP3 to potentially target as T_{reg} immunotherapies to treat lymphoma. Similarly, Carpenter and colleagues used CRISPR/Cas9 screens to identify genes influencing macrophage viability and NF-κB signaling and, therefore, macrophage-mediated protection against infection.[39] By targeting all RefSeq annotated coding genes in a Cas9-expressing, immortalized bone marrow-derived macrophage cell line containing an NF-κB-GFP reporter, they identified 115 novel regulators of NF-κB signaling in macrophages. These positive and negative regulators may serve as therapeutic targets to tune inflammatory responses that go awry in disease. The versatility of CRISPR/Cas9 screens to pursue questions in all hematopoietic cells allows for the continuous and more rapid investigation of genes regulating hematopoiesis at the HSC level and beyond. This important new ability to tease apart complex regulatory networks that dictate HSC differentiation and mature cell function will enable more precise control of HSC function for therapeutics by defining gene targets for manipulation.

3.2 | CRISPRi screens

Hematopoiesis requires the regulated and timed expression of multiple genes, orchestrated by complex interactions between gene promoters and non-coding regulatory elements called enhancers.[40–43] This is not only true during adult steady state hematopoiesis, but also critical for the proper development of the hematopoietic system during gestation.[44] Since enhancers regulate gene expression, they are key to understanding how to manipulate HSC function for therapeutic use and provide novel therapeutic targets. Although it is usually clear which promoter correspond to which gene, it remains more challenging to pair enhancers to specific genes. This gap is in part due to the physical separation between many enhancers and their target genes. Unlike promoters, which are ~35 base pairs upstream of a transcription start site, a gene can be regulated by a single or multiple enhancers located thousands of bases away from the transcription start site.[45] This lack of understanding of enhancer-promoter relationship is further complicated by the fact that some enhancers are functionally redundant, while others regulate multiple genes. As HSC multipotency and cell fate decisions depend on enhancer-mediated epigenetic regulation, there is a need to systematically test individual putative enhancers by functional assays.

Enhancers can be identified by CRISPRi, as demonstrated by the efficient silencing of globin genes by CRISPRi targeting of the HS2 enhancer.[46] Engreitz and colleagues capitalized on a high throughput CRISPRi screen to survey enhancer activity of sequences surrounding the loci of the transcription factors GATA1 and MYC.[47] By designing sgRNA libraries tiled across 74-kilobase (kb) and 1.2 mega base regions, the authors identified two enhancers for GATA1 and seven enhancers for MYC. Interestingly, they found little correlation between their results and putative enhancers previously identified by DNase I hypersensitive sites (DHS) or H3K27ac modifications. In particular, many of the previously predicted regions did not have enhancer function. This underscores the ineffectiveness of these

methods to map non-coding regulatory regions and, conversely, the power of CRISPRi-based functional approaches in linking operational enhancer-gene pairs in HSCs.

Recently, Shendure and colleagues developed a framework for interrogating functional enhancer-gene relationships by designing a powerful and unbiased CRISPRi-based enhancer screen. Their motivation for undertaking these large-scale, sequencing heavy experiments was to rapidly delineate the function of the over one million candidate regulatory elements identified in the human genome.[48] The study targeted candidate enhancers by several criteria including open chromatin, histone modifications and RNA Pol II occupancy. To individually test the function of each of these candidates would be incredibly labor intensive. Instead, the team harnessed the power of CRISPR-based genomic screens to test 5779 candidate enhancers in a single experiment. The authors designed sgRNA libraries for these candidates in K562 erythroleukemic cells, a hematopoietic cell line. These screens were particularly unique because they introduced guides at a high multiplicity of infection (MOI), resulting in an average of 28 guides per cell. The power of these experiments is evident. The use of the high MOI was calculated to be the equivalent of profiling 5.8 million single cell transcriptomes with an MOI of 1. Of the 5779 candidate enhancers, 664 of them were successfully paired to genes, an incredible achievement from a single screen. This would be an ideal framework for matching HSC enhancer-gene pairs, as HSC rarity makes them more difficult to procure and manipulate.

An important consideration with CRISPRi is how KRAB-induced heterochromatin may spread to neighboring regions of the target loci. H3K9me heterochromatin marks have been observed 4.5 kb to tens of kilobases away from the guide target site, suggesting that long-range interactions of the target site with other regions via 3-D chromatin architecture can induce off-target heterochromatin formation.[46,49] Therefore, interpretation of such experiment must be made with the utmost caution and require further validation of identified enhancers by other methods. This will ensure phenotypes are indeed due to the targeted sequence and not heterochromatin spreading.

As highlighted here, CRISPR-based screens are incredibly versatile. While they can be labor and resource intensive, they allow for systematic interrogation of multiple genes and genetic perturbations of both the coding and non-coding genome in a single experiment. The advantage of such approaches is they are very high-throughput and generate vast amounts of data, often revealing new questions to pursue and hypotheses to test beyond their original purpose. Limitations include potential for both false-positive and false-negative hits, the extent of which depend on several factors, including sequencing depth. Additionally, as CRISPR-based screens rely heavily on sequencing technologies, they require development of methods to analyze and interpret the results, which may present barriers to users. The design of guide RNAs continues to improve guide efficiency and limit off-target effects,[50] validation of hits using complementary functional approaches is still necessary. While there are limitations to CRISPR-based screens, they are becoming a more commonplace tool in hematopoiesis research as they are powerful, high-throughput hypothesis-generating approaches.

4 | NOVEL CRISPR-BASED LINEAGE-TRACING ANIMAL MODELS

An important application of CRISPR in hematopoietic research has been the development of novel lineage tracing animal models to delineate hematopoietic development and differentiation more accurately. Junker and colleagues employed CRISPR/Cas9 as a tool to create biological barcodes.[51] Without a template for repair, Cas9 will induce indels at the DNA site complementary to the sgRNAs. Spanjaard et al. took advantage of these random mutations to create a barcode, or "CRISPR scar", and used this approach to target a fluorescent protein in a transgenic zebrafish called LINNAEUS (LINeage tracing by Nuclease-Activated Editing of Ubiquitous Sequences). They used these scars along with single cell transcriptomes to identify all major classes of cell types and further track the development of the hematopoietic system from the endothelial lineage. Interestingly, these data corroborated previous evidence for a vascular origin of HSCs.[52,53]

Similar to Spanjaard et al, Camargo and colleagues developed a murine CRISPR-based lineage tracing model that relied on CRISPR-induced barcodes. They engineered a mouse model with a 276 basepair Cas9-target nucleotide array and doxycycline inducible Cas9, allowing for the precise temporal expression of Cas9 and generation of up to 44 000 unique barcode sequences.[54] Using this method called CARLIN (CRISPR Array Repair LINeage tracing), Bowling et al. was able to reconstruct the developmental hematopoietic tree. It is thought that the hematopoietic system is established in early development during multiple waves of hematopoiesis from transient progenitors.[55–57] Little is known about the clonal properties of those early progenitors, many of which contribute to life-long immunity. These progenitors seed the bone marrow, and give rise to postnatal HSCs and progenitors. Using the CARLIN system, the authors uncovered that only a few of the HSC clones that are immunophenotypically considered "definitive" (Lin-Kit+Sca1+CD150-CD48-) actually migrate to the bone marrow and give rise to life-long hematopoiesis. Therefore, the non-migrating clones may in fact represent coexisting pools of fetal HSCs that represent transient developmental waves of hematopoiesis such as the developmentally restricted (dr)HSCs discovered by Beaudin et al.[58,59]

The CRISPR/Cas9 lineage tracing models described above are forging the path for more comprehensive and sensitive analysis of lineage relationships during developmental and definitive hematopoiesis. These models complement the many decades of transplantation-based hematopoietic interrogation and allow analysis of fate decisions under minimally perturbed conditions in situ. The limitations of these models to infer cause and consequence of differentiation highlight the need for the generation of additional CRISPR/Cas9 models to answer outstanding questions in hematopoiesis research. For example, models to address the in vivo role of putative enhancers would greatly

add to our understanding of HSC regenerative capacity throughout life with the potential to translate such findings into the clinic.

5 | MODELING HEMATOLOGICAL DISEASES

In addition to answer questions on normal hematopoiesis, CRISPR has also made it more feasible to model malignant and non-malignant hematopoietic disorders in animal models. For example, mixed lineage leukemia (MLL) gene rearrangements are the most common causative genetic mutations of infant leukemias and represent very aggressive disease that is difficult to treat. Unfortunately, there are no faithful animal models of MLL rearrangements, as many of them do not recapitulate the oncogenic properties seen in humans. Therefore, there is a great need to develop accurate animal models of MLL-induced leukemias to enable better design of therapeutics to treat these aggressive diseases. Schneidawind and colleagues developed such a model for MLL-AF9-induced leukemia using human cord blood HSPCs.[60] They used CRISPR/Cas9 to generate an MLL-AF9 cell line by designing guides for the MLL and AF9 loci and co-transfecting human umbilical cord cells with both guides and Cas9 protein. The CRISPR-manipulated cells were used to test the synergistic effects of two drugs, DOT1L and PRMT5 inhibitors, currently being clinically tested (NCT01684150, NCT02783300 respectively).[61] In clinical trials, these drugs alone were minimally effective, despite showing great promise in pre-clinical trials. The authors demonstrated that the combination of these two drugs had synergistic anti-tumor effects. One caveat is that this is an in vitro system; therefore, an important next step is to use this system in vivo, possibly by crossing Cas9-expressing mice with MLL/AF9 sgRNA expressing mice to induce leukemia.

6 | CHALLENGES AND SOLUTIONS FOR IMPLEMENTING CRISPR IN HEMATOLOGIC DISEASE

While being able to model diseases is an important step in understanding human hematological diseases, the ultimate goal is to translate this technology into therapeutics. Despite great advances in CRISPR technology, there are still limitations that must be overcome to use CRISPR/Cas9 effectively and safely in humans. Primarily, the introduction of an active endonuclease with uncertain off-target effects is of great concern. As efforts to detect and mitigate undesired cutting are rapidly developing,[62,63] unfortunately, new hurdles are continually discovered. Some researchers have detected the chromosome shattering syndrome referred to as "chromothripsis" upon CRISPR-editing,[64] and DNA damage response pathways can be activated in human HSCs due to Cas9 cleavage of DNA.[65] It is especially difficult to treat genetic disorders that require a template for homology-directed repair, because this requires the successful delivery of three components (Cas9, sgRNA and template). Genetic editing platforms that do not induce double stranded breaks are therefore being explored. As previously mentioned, one such system is base editing.[18,19] If a single nucleotide mutation is the genetic lesion, there is a 33% possibility the substitution will be correct (even higher if the codon is restored in protein coding genes). Bauer and colleagues used base editing in human peripheral blood-mobilized CD34+ cells to disrupt the enhancer of BCL11A, a transcriptional repressor of fetal hemoglobin (HbF).[66] Excitingly, the authors observed induction of HbF expression in adult erythroid lineage cells when both BCL11A alleles were successfully edited. The authors then performed the same experiment on cells from sickle cell patients and observed similar induction of HbF expression upon base editing of the BCL11A enhancer. This was sufficient to prevent sickling of red blood cells derived from these edited HSPCs. Importantly, edited HSPCs were capable of long-term multilineage reconstitution, as determined by serial transplantation, indicating a robust technique to potentially cure patients with beta-globin disorders. This proof of principle experiment is an important step toward translating base-editing into a cure for sickle cell anemia.

Another obstacle of using CRISPR/Cas9 systems in hematopoietic cells is the delivery of the Cas9 protein. In mice and other model organisms, the generation of CRISPR/Cas9 transgenic lines can remove the barrier of Cas9 delivery. Mice expressing Cas9 from the H11 locus,[67] Cas9-GFP from the Rosa26 locus,[68] dox-inducible Cas9 from the Col1A1 locus,[69] dCas9-SunTag (transcriptional activator) from the Rosa26 locus,[70] and dCas9-KRAB from the H11 locus[71] are some example of transgenic CRISPR mouse models that have already been generated (Table 1). These mouse models only need delivery of the sgRNA to harness the power of their CRISPR machinery. It is increasingly straightforward to make transgenic models by using CRISPR technology, so the different "flavors" of CRISPR/Cas9 mouse models will continue to diversify. Importantly, the academic convention of sharing these tools is greatly accelerating progress toward the generation of more CRISPR technologies.

For clinical HCT (hematopoietic cell transplantation), CRISPR components must be delivered to and transiently functional in HSPCs. HSPCs are notoriously difficult to transfect or transduce, and difficult to maintain or expand in culture without loss of reconstitution potential.[72,73] Additionally, Cas9 is large (~1400 amino acids), pushing the size limit of most delivery systems for HSPCs, like adeno-associated viruses (AAVs). Several recent reviews of different delivery methods of the CRISPR/Cas machinery to cells, including physical, viral, non-viral and ribonucleoprotein strategies, have been published.[74–77] Use of smaller Cas variants would greatly ameliorate delivery problems associated with size. The most recent engineered Cas variant, Cas12f, is almost half the size of Cas9 and aptly named CasMINI[78] and is compatible with mammalian cells. These advances lower the hurdles of HSC editing.

7 | CURRENT CLINICAL APPLICATIONS OF CRISPR-EDITED HSCs

Exciting progress has been made toward CRISPR-based autologous HCT to treat and cure hematological diseases (Figure 2).[88] Although

TABLE 1 Examples of transgenic mouse models expressing cutting or bioengineered Cas9 proteins

Mouse model	Functionality	Locus	Reference
Cas9	Endonuclease	H11	Chiou et al.[67]
Dox-inducible Cas9	Inducible endonuclease	ROSA26	Katigbak et al.[69]
Cas9-GFP	Target sequence location	ROSA26	Platt et al.[68]
dCas9-SunTag	CRISPRa	Col1A1	Wangensteen et al.[70]
dCas9-KRAB	CRISPRi	H11	Oguri et al.[71]

Abbreviations: CRISPRa, transcriptional activation; CRISPRi, transcriptional interference; GFP, green fluorescent protein.

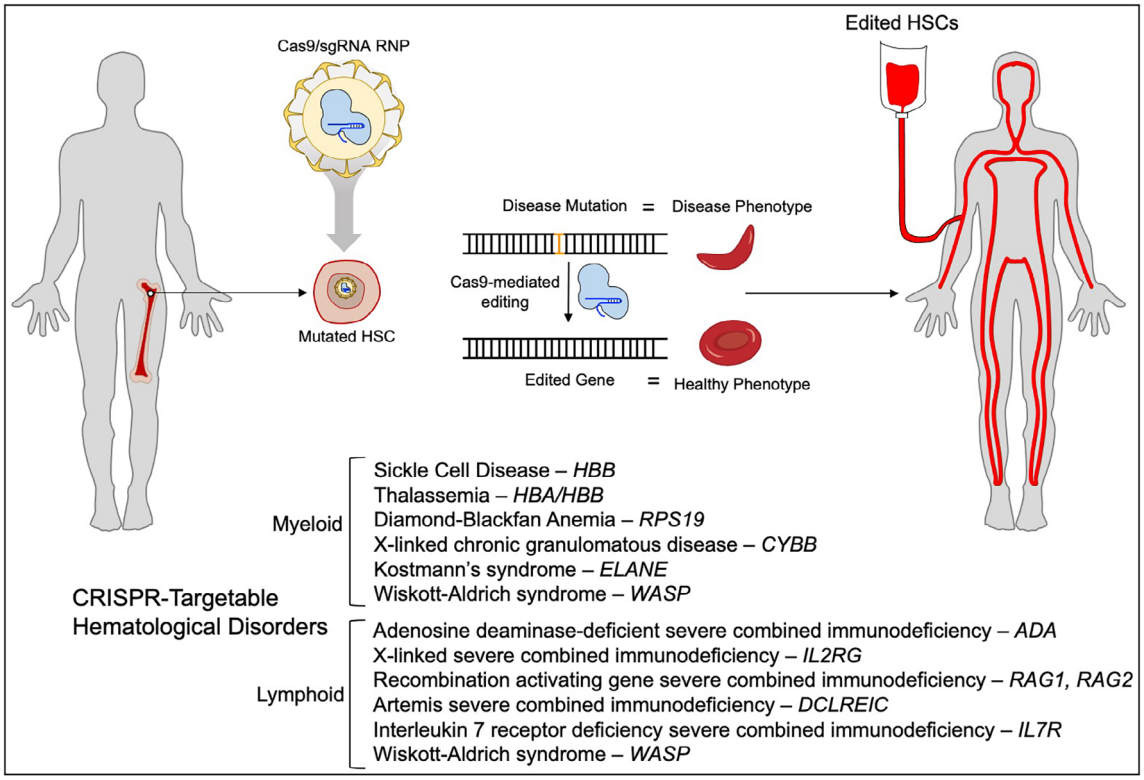

FIGURE 2 Clinical application of CRISPR/Cas9 editing. CRISPR/Cas9-based editing of HSCs to treat hematological disorders like sickle cell disease can cure patients without having to find suitable matched donors. HSCs are isolated from disease patients and Cas9 and guide are delivered as either ribonucleoprotein complexes with sgRNAs or as plasmids. HSCs are tested for effective editing and then re-introduced to the conditioned patient. Multiple hematological disorders are caused by known, single-gene mutations (Disease – *GENE*) that affect myeloid and/or lymphoid cells and may be suitable for CRISPR-editing in HSCs. *HBA/HBB*[79]; *RPS19*[80]; *CYBB*[81]; *ELANE*[82]; *WASP*[83]; *ADA*[84]; *IL2RG*[85]; *RAG1/RAG2*[86]; *DCLREIC*[87]; *IL7R*.[86] CRISPR, clustered regularly interspaced short palindromic repeats; HSC, hematopoietic stem cell [Color figure can be viewed at wileyonlinelibrary.com]

allogenic HSC transplantations are a standard therapeutic for several hematological diseases, they have significant limitations. First, the number of patients in need of HCT far exceeds the number of suitable, matched donors. Secondly, there is always a risk of graft-vs-host disease and complications arising from undergoing immunosuppressive regimens for transplantation. For many patients, autologous transplantation combined with gene editing is therefore preferred strategy over allogeneic transfer[89] and CRISPR technologies have further shifted the balance toward genetic correction of autologous HSCs. Previous efforts to correct genetic disorders for autologous HCT used viral vectors for gene therapy. However, early gene therapy trials were marred by the activation of proto-oncogenes and have only recently regained traction in clinical trials.[90] The advent of CRISPR as a more precise and efficient genome editing tool has reignited gene therapy and therefore become a viable method to correct disease HSCs for autologous transplants. A number of clinical trials are already underway to treat sickle cell anemia, beta-thalassemia, acute myeloid leukemia (AML), and HIV (Table 2) and some have already shown promising results in the few patients enrolled.[64,91–94] These therapies all take a similar approach: hematopoietic cells—containing HSCs as the long-term "active ingredient"—harboring deleterious mutations are harvested from patients, then CRISPR machinery is delivered to the cells to fix the mutation, and finally edited cells are returned to a conditioned patient (Figure 2). In the

TABLE 2 A non-comprehensive reference to examples of clinical trials of CRISPR-edited hematopoietic cells to be used for transplantation

Trial number	Target locus	Indication	Responsible party
NCT04774536	β-globin	Sickle Cell Disease	Mark Walters, MD, Professor in Residence, University of California, San Francisco
NCT04819841	β-globin	Sickle Cell Disease	Graphite Bio, Inc.
NCT03745287	BCL11A	Sickle Cell Disease	Vertex Pharmaceuticals Incorporated
NCT03728322	β-globin	Beta thalassemia	Allife Medical Science and Technology Co., Ltd.
NCT03655678	BCL11A	Beta thalassemia	Vertex Pharmaceuticals Incorporated
NCT03164135	CCR5	HIV-1	Chen Hu, Study Director, Affiliated Hospital to Academy of Military Medical Sciences
NCT04849910	CD33	AML	Vor Biopharma

Note: Information from clinicaltrials.gov.

case of sickle cell anemia, two approaches to fix the unhealthy HSCs have been taken. One is to re-activate the fetal hemoglobin gene by targeting its repressor, BCL11A (NCT03745287). The other is to replace the mutated β-globin with a repaired version (NCT04774536, NCT04819841). With regards to AML, CRISPR-based strategies have been designed to target CD33 on HSC and progenitor cells to allow for more effective anti-CD33 CAR-T or antibody therapy (NCT04849910). Additionally, in an effort to make immune cells resistant to HIV infection, CCR5 is being targeted by CRISPR in CD34+ HSPCs since CCR5 serves as an essential coreceptor of HIV infection (NCT03164135). A few pre-clinical studies have also demonstrated proof of concept CRISPR-based therapies to treat severe combined immunodeficiency (SCID) and chronic granulomatous disease (CGD) by targeting IL2RG and CYBB, respectively, in HSCs.[95–97] Analogous strategies will likely follow for the lymphoid regulator IL7R[86,98] and the other 170 single gene mutations known to impair human hematopoiesis.[99] Due to the life-long persistence of transplanted HSCs, these strategies are potential single-treatment, permanent cures. We expect that this is just the beginning of life-saving CRISPR-edited HSC therapies.

8 | FUTURE OF CRISPR IN HEMATOPOIESIS

With each new technological advancement, there is a corresponding advancement of biology. The number of resource papers published in the last 5 years using CRISPR-based screens and other genome editing techniques indicate an upcoming surge in research articles using these methods. Many outstanding questions in hematopoiesis are now attainable using the variety of high-throughput screens, novel transgenic CRISPR animal models, and engineered CRISPR components mentioned here. In particular, these new experimental techniques may lay to rest some of the most hotly debated subjects in field. What are the origins of fetal HSCs? How plastic is HSC lineage potential throughout development and life? Is hematopoietic aging manifested by HSCs or progenitors? In addition to definitive answers, these methods will be fundamental to generating new hypotheses to further advance both experimental and clinical hematology.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

This review does not present any data. Follow references for specific data availability.

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