In recent years, tremendous advances have been made in the use of gene editing to precisely engineer the genome. This technology relies on the activity of a wide range of nuclease platforms — such as zinc-finger nucleases, transcription activator-like effector nucleases, and the CRISPR–Cas system — that can cleave and repair specific DNA regions, providing a unique and flexible tool to study gene function and correct disease-causing mutations. Preclinical studies using gene editing to tackle genetic and infectious diseases have highlighted the therapeutic potential of this technology. This review summarizes the progresses made towards the development of gene editing tools for the treatment of haematological disorders and the hurdles that need to be overcome to achieve clinical success.

Introduction

Genome engineering has endowed the scientific community with the ability to artificially modify genetic information, unlocking the potential of traditional medicine to new therapeutic approaches. Gene editing represents a platform in which programmable DNA nucleases specifically recognize a target genomic sequence where they introduce permanent genetic modifications. Four major classes of targeted nucleases have been described so far: meganucleases, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the CRISPR/Cas9 system (reviewed in [1]). These nucleases are designed to create double-strand breaks (DSBs) into target DNA sequences, triggering the activation of two major endogenous cellular repair mechanisms: non-homologous end joining (NHEJ) and homologous recombination (HR) [2]. NHEJ is efficient but error-prone, thus repair of the break site results in small DNA insertions or deletions (indels) [3]. Indels can disrupt target genes by shifting the reading frame or introducing stop codons, with consequent production of non-functional proteins. In contrast, HR results in accurate repair through the use of an undamaged DNA template with homology to the sequence flanking the DSB. Each of these pathways — NHEJ or HR — could potentially be exploited for therapeutic purposes. CRISPR/Cas9-based genome editing has been widely used in proof-of-principle preclinical studies holding great promise for further clinical applications. This review will focus on the application of precise editing to treat monogenic disorders affecting the haematopoietic system and on the factors that will determine its success in the clinical setting.

Therapeutic genome editing

For many haematological conditions, such as primary immunodeficiencies and haemoglobinopathies, the only curative treatment is histocompatibility leukocyte antigen (HLA)-matched allogeneic haematopoietic stem cell transplantation (HSCT), which replaces defective haematopoietic lineages with functional cells. The limitations of this procedure include donor availability with the consequent risk of graft rejection, incomplete immune reconstitution, graft-versus-host disease, death and/or long-term dysfunction arising from the conditioning regimen [4,5]. Transplantation of autologous, genetically modified stem cells could represent an alternative to allogeneic HSCT, and many groups have worked over the
last few decades toward achieving efficient and safe gene transfer to haematopoietic stem cells (HSCs). In order for gene therapy to be a viable and potentially life-long treatment, it is necessary to (1) correct a sufficient amount of long-term repopulating HSCs, (2) achieve a stable and regulated expression of the therapeutic gene, and (3) ensure that the process is safe. Pioneering gene therapy approaches for severe combined immunodeficiency disorders (SCID) using retroviral vectors have demonstrated the applicability of the technology to treat rare genetic diseases affecting the haematopoietic system [6]. However, viral vectors carry a potential risk of genotoxicity due to their semi-random integration pattern and to unregulated transgene expression in target cells [7,8]. Using autologous genetically modified haematopoietic stem and progenitor cells (HSPCs), gene editing could represent an alternative to conventional gene therapy and overcome some of its limitations. Engineered endonucleases that introduce DSBs at specific sequences in the genome offer much more control over viral vector site integration; moreover, the site-specific insertion of DNA or correction of a disease-causing mutation in situ guarantees that physiologically regulated gene expression is preserved. There are different gene editing applications based on the two main repair mechanisms utilized by the target cells to correct the DSB. HDR (homology-directed repair) can be used to either insert a gene into a specific ‘safe harbour’, into its own locus or to specifically repair small or point mutations in the defective gene. In contrast, if NHEJ takes place, the generated indels could abolish the expression of the protein, or the function of a regulatory region, and thus this repair pathway can be utilized to treat those diseases for which mutating a genetic element may result in clinical benefit [9].

**HDR-mediated genome editing**

**Site-specific gene correction**

Site-specific correction of disease-causing mutations represents the most straightforward approach to repair a faulty gene responsible for a monogenic disorder. In situ correction has the advantage of preserving endogenous regulatory regions and physiological gene expression, which would be particularly advantageous when targeting tightly regulated genes. Conditions for which a single or predominant mutation underlies the disease would seem to be the most amenable to this approach (Figure 1). An example is sickle cell disease (SCD). SCD is an autosomal recessive disorder affecting millions of people worldwide and is caused by an A-to-T point mutation in the sixth codon of the β-globin gene (HBB), resulting in the production of a defective globin that confers a hook/sickle shape to red blood cells [10]. ZFNs and CRISPR/Cas9 together with a donor template delivered via integration-defective lentiviral vectors (IDLV) or single-stranded DNA oligonucleotides (ssODN) have been extensively used to correct the SCD-causing mutation in different cell types [11–14]. In clinically relevant cells such as HSPCs, ZFN-mediated correction of the SCD mutation was achieved in up to 40% of the cells [15–17]. However, the frequency of correction dropped when cells were transplanted into immunodeficient mice, with only ~0.2–2.3% of engrafted long-term repopulating HSPCs harbouring a corrected copy of the HBB gene at more than 12 weeks after transplantation, thus achieving rates of editing far below the level of therapeutic relevance. To overcome this limitation, Dever et al. deployed a strategy that allows the enrichment of edited HSPCs, by including a selectable marker into the HDR donor cassette delivered via a serotype 6 adeno-associated viral vectors (AAV6). Using this approach, the authors showed that >97% of cells engrafted into immunodeficient mice were gene targeted, a significant increase compared with mice transplanted with unselected gene-edited HSPCs (~3%). Despite the increase in HDR rate, selection of targeted HSPCs before transplantation yielded an overall lower engraftment rate and recovery of fewer cells compared with standard protocols, indicating that further improvements in the selection technique and cell culture conditions are required before translating this strategy into the clinics. Site-specific genome editing has also been attempted by the group of Harry Malech for the treatment of chronic granulomatous disease (CGD). CGD is a life-threatening disease caused by mutations in any of the five subunits (gp91phox, p22phox, p40, p47phox, p67phox) that comprise the phagocyte nicotinamide adenine dinucleotide phosphate oxidase (NADPH) complex. Apart from gp91phox, which is encoded by the CYBB gene located on the X chromosome, the remaining mutated subunits lead to an autosomal recessive form of the disease. While mutations are scattered across the genes for all other CGD patient phox genes, >80% of p47phox. CGD patients are homozygous for a two-nucleotide deletion in exon 2 of the NCF1 gene, resulting in a codon frameshift and abrogation of p47phox expression [18]. Interestingly, the same mutation is found in the NCF1 pseudogenes NCF1B and NCF1C. By delivering ZFNs and an AAV6 containing a correct exon 2 sequence as a template for HDR, Merling et al. replaced the mutated exon in the NCF1 locus as well as in its pseudogenes, restoring oxidase function in 6% of myeloid cells differentiated from patient’s derived p47-CGD HSPCs and showing, for the first time, that rescue of a pseudogene function can correct a monogenic disorder.
In parallel, De Ravin et al. developed a CRISPR–Cas9 system to repair a point mutation in the CYBB gene, which is responsible for 6% of the cases of X-linked CGD (X-CGD), using a ssODN as a template to promote HDR-mediated gene repair. Seamless repair of the missense mutation restored gp91phox protein expression and function in X-CGD HSPC-derived myeloid cells, and the amount of corrected cells decreased by <50% after transplant into immunodeficient mice [20].

Site-specific gene insertion

One potential issue with site-specific gene correction is that the majority of genetic diseases are caused by mutations spanning across the genes, thus requiring tailoring of gene editing reagents for each individual patient. A more universal and attractive strategy would be to target an entire gene cassette to the desired locus so that, once integrated, the transgene would functionally correct all disease-causing mutations. To this aim, a whole expression cassette, including regulatory elements, can be inserted into a ‘safe harbour’ genomic region that is able to accommodate the expression of newly integrated DNA without adverse effects on the host cell or organism.
Therapeutics using ZFNs to target this region have demonstrated the therapeutic and clinical potential of the groups have shown that knock-out of BCL11A erythroid-specific regulatory elements. Although this strategy is specific for each single gene/disease, it could be particularly amenable for conditions in which endogenous gene regulation is essential (Figure 1).

Pioneering work by Urnov et al. showed that it is possible to correct a mutation responsible for the X-linked severe combined immune deficiency (SCID-X1) in primary cells using ZFNs directed against the IL-2 receptor common gamma-chain gene (IL2RG). By designing a donor template DNA carrying an exon 5 fragment of IL2RG, the authors reported up to 5% of HDR-mediated editing in primary T cells, paving the way for potential gene correction applications, aimed to treat SCID-X1 [22]. However, the modest rates of editing achieved in primary cells highlighted the limitation of plasmid transfection to deliver the nucleases and the HDR template, in particular when applied to cells sensitive to genetic manipulations such as HSPCs. Lombardi and colleagues first addressed this issue and managed to successfully insert a donor cassette downstream of the IL2RG promoter or in the safe harbour CCR5 locus, using an IDLV to deliver ZFNs and the HDR template to lymphoblastoid primary cells, embryonic stem cells and HSPCs. While this represented an improvement of the editing protocol for primary cells, knock-in rates, especially in therapeutically relevant HSPCs, were extremely low [23]. To overcome the poor integration level observed in repopulating stem cells, the same group then optimized various culture conditions, timing and delivery route of the reagents to enhance the insertion of an IL2RG cDNA cassette into the AAVS1 or the IL2RG locus in HSPCs derived from healthy or SCID-X1 donors, achieving up to 11% of gene targeting [24]. More recently, two preclinical studies have shown significant advancement in the correction of the IL2RG locus, by knocking in a full-length IL2RG cDNA delivered by an AAV6 donor vector using either the ZFN or CRISPR/Cas9 system [25,26]. Both groups were able to reach therapeutic levels of IL2RG expression in wild-type and SCID-X1 HSPCs, as well as in the more primitive population of HSCs, with in vitro and in vivo rescue of multi-lineage developmental potential from corrected cells. SCID-X1 represents an ideal target for proof-of-concept gene editing studies, as the tremendous selective advantage that functionally corrected cells have over mutated ones in a SCID setting [27] can compensate for the relatively low rate of HDR-mediated correction in HSPCs. Additional protocol optimization may be required to increase the percentage of gene correction in long-term repopulating stem cells to revert the disease phenotype in blood disorders where such a strong selective advantage is missing.

**NHEJ-mediated genome editing**

Although gene correction might seem the most immediate approach to therapeutic genome editing, the first clinical trial using targeted nucleases in human patients has relied on NHEJ-based genetic disruption. One advantage of this strategy is that NHEJ trends to be a more active repair pathway compared with HDR, particularly in quiescent stem cells [28]. Another benefit of NHEJ over HDR is that it relies solely on targeting nucleases with no need for designing and producing a donor template, thus making the whole process less laborious and more efficient for a potential clinical application. The first-in-human genome editing trial (NCT01044654) used ZFNs in autologous T cells to target the HIV co-receptor CCR5 [29], with the aim to mimic naturally occurring mutations that abolish its expression and engender resistance to HIV infection [30]. The clinical outcome indicated that this NHEJ-based approach was safe for patients and able to confer a survival advantage in vivo against HIV. Preclinical in vitro and in vivo studies demonstrated the feasibility and safety of ZFN-based CCR5 disruption also in HSPCs, providing a potentially life-long treatment to HIV-infected patients [31]. The technology is currently being evaluated in an ongoing clinical trial (NCT02500849) as a collaboration between City of Hope Medical Center and Sangamo Therapeutics.

The indels generated by NHEJ repair may be useful for disrupting not only coding sequences but also non-coding regulatory elements. For example, mutation of the erythroid-specific enhancer of BCL11A has been shown to increase fetal haemoglobin (HbF) expression and treat haemoglobinopathies. From the observations of new-born babies with β-globin defects [32], it is clear that by maintaining HbF expression it is possible to reduce or totally abolish the symptoms related to SCD or β-thalassemia [33–35]. BCL11A plays a crucial role in HbF transcriptional repression [36,37], in accordance with the rescue of therapeutically relevant levels of HbF expression in BCL11A knock-out murine models [38,39]. By using different gene editing platforms, many groups have shown that knock-out of BCL11A erythroid-specific enhancer restores high HbF levels, without compromising cell viability or function [38,40,41] (Figure 2). Preclinical studies made by Sangamo Therapeutics using ZFNs to target this region have demonstrated the therapeutic and clinical potential of the
approach, obtaining engraftment of modified long-term HSCs in vivo and persisting production of high levels of HbF in animals [42–44]. At the beginning of the year, a Phase 1/2 clinical trial has started to evaluate the safety of their product in six β-thalassemia patients (NCT03432364); in parallel, CRISPR Therapeutics and Vertex Pharmaceuticals have opened a Phase 1/2 clinical trial on twelve SCD-affected subjects to test the safety of their CRISPR–Cas9 platform targeting the BCL11A enhancer in HSPCs (NCT03745287).

Apart from BCL11A, several other modulators of HBB expression have been identified as potential targets of NHEJ-based gene disruption approaches (Figure 2). In particular, it has been shown that CRISPR/Cas9-mediated knock-out of the lymphoma/leukemia-related factor (LRF), an HbF silencer [45], can increase more than five times the level of HbF expression. However, the involvement of LRF in other hematopoietic functions may impair the translation of this approach into the clinic and therefore it requires further investigation [46]. The presence of high levels of HbF in patients affected by the hereditary persistence of fetal hemoglobin (HPFH) benign condition has led several research groups to focus on genome editing strategies that could reproduce large deletions in the β-globin gene cluster and mutations in the γ-globin promoter region [47–49]. It has been shown that the disruption of HGB1 and HGB2 (γ-globin) gene promoter region inhibits the repression of HbF mediated by BCL11A or LRF reversing the globin switching [50,51]. Furthermore, natural HPFH-associated deletions and point mutations have been precisely generated in vitro in HSPCs through a CRISPR/Cas9 multiplex strategy [52,53], or a microhomology-mediated end joining microdeletion approach [54]. However, the reactivation of HbF expression at clinically beneficial levels has not been achieved so far, leaving open questions regarding the efficacy [55] and potential side effects in vivo [55] of the γ-globin silencer as a therapeutic target for gene editing.

**Epigenome and base editing**

The great versatility and flexibility of the CRISPR/Cas9 platform and the ability of Cas9 to bind DNA independently from its nuclease activity led to the development of novel tools that could overcome some of the limitations of current gene editing approaches. Indeed, a ‘dead’ catalytically inactive Cas9 endonuclease (dCas9)
Challenges of therapeutic genome editing

Site-specific genome editing has transformed the research field of biology and medicine. Not only can this technique allow the functional study of a particular gene, but it is also amenable to correction of a disease-associated mutation. Despite this exciting prospect, there remain major barriers associated with the editing-based therapeutic treatment of blood disorders that must be addressed to advance clinical applications that rely on genome editing. Some of these challenges include (1) the delivery of the editing machinery ex vivo and in vivo, (2) the ability to preserve the stemness and achieve high levels of engraftment of HSCs in vivo, (3) the identification and reduction in genome-wide off-target effects induced by the nucleases.

The main objective when delivering gene editing reagents is to promote a hit-and-run activity of the nucleases, to allow for the generation of DSBs in a short period of time, while limiting toxicity and off-target activity. For HDR-based gene addition approaches, it is also necessary to deliver the DNA donor template in a transient way, to avoid unwanted integration into non-specific regions of the genome. For decades, various delivery methods, such as electroporation, nanoparticles and viral vectors, have been used to introduce the editing reagents into cells for ex vivo or in vivo approaches. For ex vivo applications, transfection of plasmid DNA is nowadays the least used technique to target primary cells, as reports indicate induction of high toxicity, off-target insertion and host immune response [66,67]. These unwanted effects are observed minimally when delivering nucleases as mRNA or, in the case of Cas9 and the gRNA, in the form of a ribonucleoprotein complex [16,68–70]. Unlike electroporation, viral vectors, such as recombinant AAV and IDLV, have been applied in both ex vivo and in vivo preclinical studies. In addition to the delivery of editing reagents, their non-integrative properties allow them to be used to provide the donor template for HDR-based gene correction. The relatively low toxicity and non-integrative nature of AAV, together with its ability to achieve high recombination frequencies with small homology regions, have made them desirable genetic tools for efficient human HSPC gene editing [25,71–73]. Despite being safe, one of the drawbacks of AAV is their limited cargo capacity of 4.5 kb that restricts the expression of large transgenes, although it has been recently reported the integration of a cassette with up to 6.5 kb in size using a multiplexing strategy [74]. Delivery through lipid- and gold-based nanoparticles is considered safer in vivo compared with the use of viral vectors, but the targeting efficiency is still below therapeutic levels [75,76].
As outlined at the beginning of this review, long-term repopulating HSCs are the ideal target for gene editing of various types of inherited haematological conditions [16]. The therapeutic benefit of gene-corrected HSCs depends on their capacity to engraft and provide long-term production of healthy blood lineage progenitors while maintaining renewable stem cells in transplanted patients. One of the hurdles associated with HSC editing is the low rate of HDR than can be achieved, mainly due to the fact that NHEJ is preferentially utilized to correct DSBs in non-dividing cells. To overcome this problem, various groups have tried either pharmacological or genetic inhibition of NHEJ [77–80], strategies to increase the rates of HDR [81,82] and synchronization of the cell cycle [83], as well as optimization of delivery conditions to enhance knock-in efficiencies. Moreover, strategies to in vitro expand primitive HSC while preserving their stem cell qualities have been put in place, with the final aim to advance efforts at HSC modification, engraftment and long-term repopulation in in vivo xenotransplantation models [24,72,84].

The intrinsic specificity of the editing machinery permits researchers to modify their chosen gene of interest at a particular locus; however, unintended off-target cleavage at different genomic sites might occur. Being a permanent genetic modification, off-target cleavage could introduce unwanted mutations which may ultimately lead to cancer, posing a huge risk for clinical therapeutic applications involving engineered nucleases. Therefore, evaluation of off-target mutagenesis is an important preclinical criterion that must be taken into account before starting clinical studies. Several off-target detection methods have been developed to assess the safety of gene editing reagents. Early developed approaches have used computational predictions to identify a limited set of genomic regions that show homology with the target site, which can be then examined for a-specific cleavage by deep sequencing [85]. More recently, unbiased methods have been proposed that allow for genome-wide assessment of off-target mutagenesis, and in certain cases can also identify gross chromosomal rearrangements [86–89]. However, these methods do not always reliably pinpoint all potential off-targets and often show a limited sensitivity. To complicate the scenario, one must take into account the baseline level of mutagenesis that exists in normal somatic tissues or that can be caused by cell expansion during the manufacturing process. Dosage and expression pattern of the nucleases, as well as cell number, cell type and features of the genomic target site, may also affect editing specificity. It is difficult to predict the impact that modifications at different genomic sites may have on cell fitness, considering that most likely the vast majority of off-target edits would be functionally neutral. Interpreting the effects of genomic perturbations is challenging and requires the identification and development of functional readouts of safety that must be tailored to the therapeutic cell type of interest. There have been several efforts to increase the specificity of genome-editing systems, such as the development of shorter gRNAs [85], nickase mutants of Cas9, Cas9–FokI fusion protein [90,91], and high-fidelity Cas9 variants [69,92]. The continued improvements of the efficiency and safety of these reagents will be essential to ensure the success of gene editing applications to treat genetic haematological disorders.

Conclusion
During the last few years, gene editing has emerged as a powerful tool for genomic functional studies and therapeutic correction of monogenic disease. Gene editing platforms, such as CRISPR/Cas9 and TALEN, have shown great promise in proof-of-principle preclinical studies to treat haematological disorders, and clinical trials using these tools are now underway. Nonetheless, there remain important challenges that need to be addressed, such as the efficiency and specificity of the editing system, especially when considering targeting of primary stem cells.

Summary
• Genome editing is a rapidly evolving technology that has particular relevance for the treatment of haematological disorders and infectious diseases.

• Blood disorders can be tackled by taking advantage of two main endogenous cell repair mechanisms — NHEJ and HDR — which lead to either disruption of genes and regulatory elements or insertion of a correct gene in a site-specific fashion.

• Despite the tremendous progress, several issues need to be addressed to make genome editing a safer and more effective therapeutic tool.
Abbreviations
AVVS1, adeno-associated virus integration site 1; BEs, base editors; CGD, chronic granulomatous disease; DSBs, double-strand breaks; HDR, homology-directed repair; HPFH, hereditary persistence of fetal haemoglobin; HR, homologous recombination; HSCs, haematopoietic stem cells; HSCT, haematopoietic stem cell transplantation; HSPCs, haematopoietic stem and progenitor cell; IDLV, integration-defective lentiviral vectors; IL2RG, IL-2 receptor common gamma-chain gene; LCR, locus control region; LRF, lymphoma/leukemia-related factor; NHEJ, non-homologous end joining; SCD, sickle cell disease; SCID, severe combined immunodeficiency disorders; SCID-X1, X-linked severe combined immune deficiency; ssODN, single-stranded DNA oligonucleotides; TALENs, transcription activator-like effector nucleases; X-CGD, X-linked CGD; ZFNs, zinc-finger nucleases.

Author Contribution
M.R., R.R. and A.C. wrote the manuscript. M.R. prepared the figures under the supervision of A.C.

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Competing Interests
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References
1  Kim, H. and Kim, J.-S. (2014) A guide to genome engineering with programmable nucleases. Nat. Rev. Genet. 15, 321–334 https://doi.org/10.1038/nrg3686
2  Liang, L., Deng, L., Chen, Y., Li, G.C., Shao, C. and Tischfield, J.A. (2005) Modulation of DNA end joining by nuclear proteins. J. Biol. Chem. 280, 31442–31449 https://doi.org/10.1074/jbc.M503776200
3  Lieber, M.R., Ma, Y., Pannicke, U. and Schwarz, K. (2003) Mechanism and regulation of human non-homologous DNA end-joining. Nat. Rev. Mol. Cell Biol. 4, 712–720 https://doi.org/10.1038/nrm1202
4  Locatelli, F., Kabbara, N., Ruggeri, A., Ghavamzadeh, A., Roberts, I., Li, C.K. et al. (2013) Outcome of patients with hemoglobinopathies given either zinc-DNA oligonucleotides; TALENs, transcription activator-like effector nucleases; X-CGD, X-linked CGD; ZFNs, zinc-finger nucleases.

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2  Liang, L., Deng, L., Chen, Y., Li, G.C., Shao, C. and Tischfield, J.A. (2005) Modulation of DNA end joining by nuclear proteins. J. Biol. Chem. 280, 31442–31449 https://doi.org/10.1074/jbc.M503776200
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2  Liang, L., Deng, L., Chen, Y., Li, G.C., Shao, C. and Tischfield, J.A. (2005) Modulation of DNA end joining by nuclear proteins. J. Biol. Chem. 280, 31442–31449 https://doi.org/10.1074/jbc.M503776200
3  Lieber, M.R., Ma, Y., Pannicke, U. and Schwarz, K. (2003) Mechanism and regulation of human non-homologous DNA end-joining. Nat. Rev. Mol. Cell Biol. 4, 712–720 https://doi.org/10.1038/nrm1202
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2  Liang, L., Deng, L., Chen, Y., Li, G.C., Shao, C. and Tischfield, J.A. (2005) Modulation of DNA end joining by nuclear proteins. J. Biol. Chem. 280, 31442–31449 https://doi.org/10.1074/jbc.M503776200
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50 Liu, N., Hargreaves, V.V., Zhu, Q., Kurland, J.V., Hong, J., Kim, W. et al. (2018) Direct promoter repression by BCL11A controls the fetal to adult hemoglobin switch. *Cell* 173, 430–442.e17 https://doi.org/10.1016/j.cell.2018.03.016

51 Martyn, G.E., Wiemert, B., Yang, L., Shah, M., Norton, L.J., Burnich, J. et al. (2018) Natural regulatory mutations elevate the fetal globin gene via disruption of BCL11A or ZBTB7A binding. *Nat. Genet.* 50, 498–503 https://doi.org/10.1038/s41588-018-0085-0

52 Ye, L., Wang, J., Tan, Y., Beyer, A.L., Xie, F., Muench, M.O. et al. (2016) Genome editing using CRISPR/Cas9 to create the HPFH genotype in HSPCs: an approach for treating sickle cell disease and β-thalassemia. *Proc. Natl Acad. Sci. U.S.A.* 113, 10661–10666 https://doi.org/10.1073/pnas.1612075113

53 Antoniani, C., Meneghini, V., Lattanzi, A., Felix, T., Romano, O., Magrin, E. et al. (2018) Induction of fetal hemoglobin synthesis by CRISPR/Cas9-mediated editing of the human β-globin locus. *Blood* 131, 1960–1973 https://doi.org/10.1182/blood-2017-10-811505

54 Traxler, E., Yao, Y., Li, C., Grevet, J., Huang, P., Wright, S. et al. (2015) Genome editing recreates hereditary persistence of fetal hemoglobin in primary human erythroid cells. *Blood* 126, 640 https://doi.org/10.1182/blood-2015-03-635532

55 Chung, J.E., Magis, W., Vu, J., Heo, S.-J., Vartivaraa, K., Walters, M.C. et al. CRISPR-Cas9 interrogation of a putative fetal globin repressor in human erythroid cells. *PLoS ONE* 14, e0208237 https://doi.org/10.1371/journal.pone.0208237

56 Deng, W., Lee, J., Wang, H., Miller, J., Reik, A., Gregory, P.D. et al. (2012) Controlling long-range genomic interactions at a native locus by targeted tethering of a loop factor. *Cell* 149, 1233–1244 https://doi.org/10.1016/j.cell.2012.03.051

57 Mendenhall, E.M., Williamson, K.E., Reyon, D., Zou, J.Y., Ram, O., Joung, J.K. et al. (2013) Locus-specific editing of histone modifications at endogenous enhancers. *Nat. Biotechnol.* 31, 1133–1136 https://doi.org/10.1038/nbt.2701

58 Xu, J., Bauer, D.E., Kereryi, M.A., Vo, T.D., Hsu, Y.-J. et al. (2013) Corepressor-dependent silencing of fetal hemoglobin expression by BCL11A. *Proc. Natl Acad. Sci. U.S.A.* 110, 6518–6523 https://doi.org/10.1073/pnas.1303976110

59 Xu, J., Bauer, D.E., Kereryi, M.A., Vo, T.D., Hsu, Y.-J. et al. (2013) Corepressor-dependent silencing of fetal hemoglobin expression by BCL11A. *Proc. Natl Acad. Sci. U.S.A.* 110, 6518–6523 https://doi.org/10.1073/pnas.1303976110

60 Gaudelli, N.M., Komor, A.C., Rees, H.A., Packer, M.S., Badran, A.H., Bryson, D.I. et al. (2017) Programmable base editing of A to T/G in genomic DNA without DNA cleavage. *Nature* 551, 464–471 https://doi.org/10.1038/nature23410

61 Gehrke, J.M., Cervantes, O., Clement, M.K., Wu, Y., Zeng, J., Bauer, D.E. et al. (2018) An APOBEC3A-Cas9 base editor with minimized bystander and off-target activities. *Nat. Biotechnol.* 36, 977–982 https://doi.org/10.1038/nbt.4199

62 Liang, P., Ding, C., Sun, H., Xie, X., Xu, Y., Zhang, X. et al. (2017) Correction of β-thalassemia mutant by base editor in human embryos. *Protein Cell* 8, 811–822 https://doi.org/10.1007/s13238-017-0475-6

63 Kim, S., Kim, D., Cho, S.W., Kim, J. and Kim, J.-S. (2014) Highly efficient RNA-guided genome editing in human cells via delivery of purified Cas9 ribonucleoproteins. *Genome Res.* 24, 1012–1019 https://doi.org/10.1101/gr.171322.113

64 Sun, L., Wu, J., Du, F., Chen, X. and Chen, Z.J. (2013) Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type-I interferon pathway. *Science* 339, 786–791 https://doi.org/10.1126/science.1232458

65 Hendel, A., Bak, R.O., Clark, J.T., Kennedy, A.B., Ryan, D.E., Roy, S. et al. (2015) Chemically modified guide RNAs enhance CRISPR-Cas genome editing in human primary cells. *Nat. Biotechnol.* 33, 985–989 https://doi.org/10.1038/nbt.3290

66 Vakulskas, C.A., Dever, D.P., Retting, G.R., Turk, R., Jacobi, A.M., Collingwood, M.A. et al. (2018) A high-fidelity Cas9 mutant delivered as a ribonucleoprotein complex enables efficient gene editing in human hematopoietic stem and progenitor cells. *Nat. Med.* 24, 1216–1224 https://doi.org/10.1038/s41591-018-0137-0

67 Hoban, M.D., Lamaquaij, D., Kuo, C.Y., Romero, Z., Long, J., Ho, M. et al. (2016) CRISPR/Cas9-mediated correction of the sickle mutation in human CD34+ cells. *Mol. Ther.* 24, 1561–1562 https://doi.org/10.1038/mt.2016.148

68 Kuo, C.Y., Long, J.D., Campo-Fernandez, B., de Oliveira, S., Cooper, A.R., Romero, Z. et al. (2018) Site-specific gene editing of human hematopoietic stem cells for X-linked hyper-IgM syndrome. *Cell Rep.* 23, 2606–2616 https://doi.org/10.1016/j.celrep.2018.04.103

69 Charlesworth, C.T., Camarena, J., Cromer, M.K., Vaidyanathan, S., Bak, R.O., Cante, J.M. et al. (2018) Priming human repopulating hematopoietic stem and progenitor cells for Cas9sgRNA gene targeting. *Mol. Ther. Nucleic Acids* 12, 89–104 https://doi.org/10.1016/j.omtn.2018.04.017

70 Dever, D.P., Bak, R.O., Reinsch, A., Camarena, J., Washington, G., Nicolas, C.E. et al. (2016) CRISPR/Cas9 β-globin gene targeting in human hematopoietic stem cells. *Nature* 539, 384–389 https://doi.org/10.1038/nature19243

71 Bak, R.O. and Porteus, M.H. (2017) CRISPR-mediated integration of large gene cassettes using AAV donor vectors. *Cell Rep.* 20, 750–756 https://doi.org/10.1016/j.celrep.2017.06.064

72 Finn, J.D., Smith, A.R., Patel, M.C., Shaw, L., Youniss, M.R., van Heren, J. et al. (2018) A single administration of CRISPR/Cas9 lipid nanoparticles achieves robust and persistent in vivo genome editing. *Cell Rep.* 22, 2222–2235 https://doi.org/10.1016/j.celrep.2018.02.014

73 Lee, K., Conboy, M., Park, H.M., Jiang, F., Kim, H.J., Dewitt, M.A. et al. (2017) Nanoparticle delivery of Cas9 ribonucleoprotein and donor DNA in vivo induces homology-directed DNA repair. *Nat. Biomed. Eng.* 1, 889–901 https://doi.org/10.1038/s41555-017-0137-2

74 Li, J., Summervill, M., Nitiss, K.C., Nitiss, J.L. and Hanakahi, L.A. (2017) TP1 is required for efficient non-homologous end joining in human cells. *DNA Repair* 60, 40–49 https://doi.org/10.1016/j.dnarep.2017.10.003

75 Yu, C., Liu, Y., Ma, T., Liu, K., Xu, S., Zhang, Y. et al. (2015) Small molecules enhance CRISPR genome editing in pluripotent stem cells. *Cell Stem Cell* 16, 142–147 https://doi.org/10.1016/j.stem.2015.01.003
79 Boitano, A.E., Wang, J., Romeo, R., Bouchez, L.C., Parker, A.E., Sutton, S.E. et al. (2010) Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. *Science* **329**, 1345–1348 https://doi.org/10.1126/science.1191536
80 North, T.E., Goessling, W., Wаликей, C.R., Lengerke, C., Kopani, K.R., Lord, A.M. et al. (2007) Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. *Nature* **447**, 1007–1011 https://doi.org/10.1038/nature05883
81 Li, G., Zhang, X., Zhong, C., Mo, J., Guan, R., Yang, J. et al. (2017) Small molecules enhance CRISPR/Cas9-mediated homology-directed genome editing in primary cells. *Sci. Rep.* **7**, 8943 https://doi.org/10.1038/s41598-017-09306-x
82 Song, J., Yang, D., Xu, J., Zhu, T., Chen, Y.E. and Zhang, J. (2016) RS-1 enhances CRISPR/Cas9- and TALEN-mediated knock-in efficiency. *Nat. Commun.* **7**, 10548 https://doi.org/10.1038/ncomms10548
83 Orthwein, A., Noordermeer, S.M., Wilson, M.D., Landry, S., Enchev, R.I., Sherker, A. et al. (2015) A mechanism for the suppression of homologous recombination in G1 cells. *Nature* **528**, 422–426 https://doi.org/10.1038/nature16142
84 Zonari, E., Desantis, G., Petrillo, C., Boccalatte, F.E., Lidonnici, M.R., Kajaste-Rudnitski, A. et al. (2017) Efficient ex vivo engineering and expansion of highly purified human hematopoietic stem and progenitor cell populations for gene therapy. *Stem Cell Rep.* **8**, 977–990 https://doi.org/10.1016/j.stemcr.2017.02.010
85 Fu, Y., Foden, J.A., Khayter, C., Maeder, M.L., Reyon, D., Joung, J.K. et al. (2013) High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. *Nat. Biotechnol.* **31**, 822–826 https://doi.org/10.1038/nbt.3101
86 Gabriel, R., von Kalle, C. and Schmidt, M. (2015) Mapping the precision of genome editing. *Nat. Biotechnol.* **33**, 150–152 https://doi.org/10.1038/nbt.3142
87 Frock, R.L., Hu, J., Meyers, R.M., Ho, Y.-J., Kii, E. and Alt, F.W. (2015) Genome-wide detection of DNA double-stranded breaks induced by engineered nucleases. *Nat. Biotechnol.* **33**, 179–186 https://doi.org/10.1038/nbt.3101
88 Tsai, S.Q., Zheng, Z., Nguyen, N.T., Liebers, M., Topkar, V.V., Thapar, V. et al. (2015) GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas nucleases. *Nat. Biotechnol.* **33**, 187–197 https://doi.org/10.1038/nbt.3117
89 Kim, D., Bae, S., Park, J., Kim, E., Kim, S., Yu, H.R. et al. (2015) Digenome-seq: genome-wide profiling of CRISPR-Cas9 off-target effects in human cells. *Nat. Methods* **12**, 237–243 https://doi.org/10.1038/nmeth.3284
90 Tsai, S.Q., Wyvekens, N., Khayter, C., Foden, J.A., Thapar, V., Reyon, D. et al. (2014) Dimeric CRISPR RNA-guided FokI nucleases for highly specific genome editing. *Nat. Biotechnol.* **32**, 569–576 https://doi.org/10.1038/nbt.2908
91 Mali, P., Aach, J., Stranges, P.B., Esveld, K.M., Moosburner, M., Kosuri, S. et al. (2013) CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering. *Nat. Biotechnol.* **31**, 833–838 https://doi.org/10.1038/nbt.2675
92 Kleinsteve, B.P., Pattanayak, V., Prew, M.S., Tsai, S.Q., Nguyen, N.T., Zheng, Z. et al. (2016) High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. *Nature* **529**, 490–495 https://doi.org/10.1038/nature16526