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CRISPR/Cas9 for the treatment of haematological diseases: A journey from bacteria to the bedside

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Abstract

Genome editing therapies represent a significant advancement in next-generation, precision medicine for the management of haematological diseases, and CRISPR/Cas9 has to date been the most successful implementation platform. From discovery in bacteria and archaea over 3 decades ago, through intensive basic research and pre-clinical development phases involving the modification of therapeutically relevant cell types, CRISPR/Cas9 genome editing is now being investigated in ongoing clinic trials. Despite the widespread enthusiasm brought by this new technology, significant challenges remain before genome editing can be routinely recommended and implemented in the clinic. These include risks of genotoxicity resulting from off-target DNA cleavage or chromosomal rearrangement, and suboptimal efficacy of homology-directed repair editing strategies, which thus limit therapeutic options. Practical hurdles such as high costs and inaccessibility to patients outside specialised centres must also be addressed. Future improvements in this rapidly developing field should circumvent current limitations with novel editing platforms and with the simplification of clinical protocols using *in vivo* delivery of editing reagents.

Keywords

CRISPR/Cas9; Genome editing; Haematopoietic stem cells; T lymphocytes; Clinical trial

Milestones of CRISPR/Cas9 research: from discovery to human therapeutics

Genome editing is now offering new hope for the cure of various congenital and acquired conditions through precise correction of aberrant genetic sequences, or by the site-specific replacement of defective genes. Biological applications of these technologies are expanding exponentially but are all based on the introduction of a double-stranded DNA break (DSB)

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at a given chromosomal locus by site-specific nucleases. Several genome editing platforms have been developed to date, including zinc finger nuclease (ZFN) and transcription activator-like nuclease (TALEN), as reviewed in (Porteus 2016), but the most widely employed is the Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) platform. This RNA-guided endonuclease has received widespread public attention, probably due to its relative ease of use. By introducing a DNA DSB at a customised genetic location and enlisting the cellular repair machinery (described in further detail in the next section), CRISPR/Cas9 is a versatile tool that can be applied to a variety of biological contexts.

DNA sequences encoding this CRISPR system were first identified in several bacterial and archaeal species in the late 1980s-early 1990s (Ishino, et al 1987), and it is now evident that over 40% of bacteria and 90% of archaea contain genomic information for this system (Mojica, et al 2005) and reviewed in (Li and Peng 2019)). In these organisms, CRISPR functions as an element of the adaptive immune system against invading phages, but this system, and particularly type II CRISPR system that only requires one protein (Cas9) for the recognition and cleavage of the target site, was subsequently adapted for use in mammalian cells. In 2012, Doudna and Charpentier showed that CRISPR/Cas9 introduces DSBs in a target sequence (Gasiunas, et al 2012, Jinek, et al 2012), and a few months later, pioneering work demonstrated that CRISPR/Cas9 could be repurposed to gene-edit human cells (Cho, et al 2013, Cong, et al 2013, Mali, et al 2013a, Mali, et al 2013b) (Figure 1). In this context, the system was reconfigured and simplified by fusing the CRISPR RNA sequence (crRNA) with the transactivating crRNA (tracrRNA) into a single 100 nucleotide molecule, named single guide RNA (sgRNA), which complexes with the Cas9 protein and guides it to the target site.

The ability of CRISPR/Cas9 to introduce a DSB in DNA at a given genomic site has opened the door to the manipulation of genes in a variety of ways, following repair of the lesion by the cellular repair machinery. The two major DNA repair pathways in mammalian cells are known as non-homologous end joining (NHEJ) or homologous recombination (commonly known as homology-directed repair, HDR) (Figure 2). Of note, a third repair pathway known as microhomology-mediated end-joining (MMEJ) also contributes to the generation of edited alleles after CRISPR/Cas9 treatment and shares components of both the NHEJ and HDR pathways (Bae, et al 2014). NHEJ is the default repair pathway in human cells and is an error-prone process because it involves the re-ligation of the two DNA ends with frequent nucleotide insertion or deletion (INDELS) at the cut site. This pathway is predominant in most cells types and can be harnessed to knock out a gene, disrupt a DNA regulatory motif (such as the binding site for a transcription factor), or create new splicing variants. One drawback of NHEJ is the production of an heterogenous population of edited cells: since a large variety of INDELS are generated, some may be undesirable for a given therapeutic application. The other major repair pathway, known as HDR, relies on a homologous donor DNA template, generally supplied along with the CRISPR/Cas9, to introduce more controlled genetic changes to the chromosomal target. This pathway involves different mechanisms that have been covered in detail elsewhere (Yeh, et al 2019) depending on whether a double- or single-stranded DNA is used, for example, with a single-stranded oligonucleotide (ssODN). HDR-editing allows for the introduction of a variety of changes in

a target sequence, such as the substitution of one or multiple nucleotides, the insertion of novel coding information spanning several hundred base-pairs, or the deletion of genetic information. Adeno-associated virus (AAV) have generally been used for the delivery of large donor DNA templates (up to 4.4kbp) whereas donor ssODN have mainly been used for the introduction of small nucleotide variants within a target sequence. As mentioned previously, while HDR is attractive for its ability to encode precise edits, it is limited by low HDR/NHEJ ratios observed in most cells, particularly in long term engrafting haematopoietic stem cells.

Together, this plethora of genomic modifications enabled by CRISPR/Cas9 makes it a versatile tool for many valuable therapeutic applications. In this review, we particularly focus on haematological diseases, first by describing pre-clinical development, then by outlining active and completed clinical trials.

CRISPR/Cas9 genome editing of therapeutically relevant target cells

Two cell types with key roles in haematological disorders and which have therefore been prominent targets for CRISPR/Cas9-editing are human haematopoietic stem and progenitor cells (HSPCs) and T lymphocytes. Ideally, treatment should result in efficient, precise, non-toxic genetic alteration without perturbation of normal physiology of these cells. This is particularly important for HSPCs, which have self-renewal properties, multipotent differentiation potential (Cornu, et al 2017, Gonzalez-Romero, et al 2019), and for which the engineered footprint is expected to propagate across all blood lineages and persist for an entire lifetime. To fulfil these goals, several delivery vehicles for transient CRISPR/Cas9 expression have been tested and optimised, including plasmid DNA, RNA and ribonucleoprotein (RNPs), consisting of the Cas9 purified protein coupled with the sgRNA. RNPs have proven to be the preferred delivery method for *ex vivo* manipulation of HSPCs and T cells due to high editing efficiency and low toxicity (Bak, et al 2018a, Lattanzi, et al 2019), likely because they do not induce an innate immune response such as that seen with mRNA or double-stranded DNA (Cromer, et al 2018). The addition of chemical modifications such as 2’-O-methyl-3’phosphorothiate on the extremities of the sgRNA have further increased editing efficiency by enhancing stability after delivery to the target cells (Hendel, et al 2015). Importantly, both the Cas9 protein and the chemically modified sgRNA are commercially manufactured and can be purchased as “off-the-shelf” reagents with stringent release criteria to ensure consistency in quality across different lots and reproducibility in editing efficiency across experiments. Below, we describe therapeutic strategies that include the correction of disease-causing mutations in monogenic disorders, the introduction of beneficial mutations for malignant and non-malignant haematological disorders, and the inactivation of viral pathogens.

HSPC-based CRISPR/Cas9 therapeutic strategies

Therapeutic genome editing of HSPCs is still in its infancy, but these cells have been targeted *ex vivo* using retroviral vectors in gene therapy applications since the late 1980s. Despite early setbacks caused by insertional mutagenesis and oncogenesis of the provirus (Hacein-Bey-Abina, et al 2008), gene replacement therapies using optimised retroviral

vectors are now being successfully applied in multiple clinical contexts that include β-haemoglobinopathies (Ribeil, et al 2017, Thompson, et al 2018), inherited bone marrow disorders such as Fanconi anemia (Rio, et al 2019) and primary immunodeficiencies (Mamcarz, et al 2019, Shaw, et al 2017). These vector-based approaches, however, are limited by the uncontrolled chromosomal integration of the therapeutic transgene, by the use of non-physiological (often suboptimal) promoters to drive expression of the transgene, and by challenges associated with large-scale, clinical-grade retroviral vector production. Genome editing can potentially offer a more precise and therefore safer alternative, and may be applicable to a wider array of diseases. Below, we focus on diseases for which HSPC CRISPR/Cas9 therapies have reached advanced stages following validation in preclinical experiments.

β-haemoglobinopathies are the most common monogenic disorder worldwide and affect the normal production of adult haemoglobin due to mutations in the β-globin gene (*HBB*). CRISPR/Cas9 editing approaches are being avidly investigated as a source of cure in these diseases. The two most common diseases are β-thalassaemia with low or absent β-globin production leading to dyserythropoiesis and severe anaemia, and sickle cell disease (SCD) with the production of a mutant form of β-globin causing polymerization of globin molecules and sickling of red blood cells in the circulation. Two main CRISPR/Cas9 strategies have been developed as attempts to treat these diseases: direct correction of the underlying mutation, or indirect correction of disease phenotype by reactivation of fetal haemoglobin (HbF). HbF has long been known to ameliorate clinical outcome in patients suffering from β-haemoglobinopathies from the study of individuals born with the benign condition known as hereditary persistence of fetal haemoglobin (HPFH) (Forget 1998).

In both approaches, HSPCs are the targets of editing to achieve long-term generation of modified erythrocytes that produce the therapeutic form of haemoglobin, thereby delivering a durable, one-time treatment that will last for an entire lifetime. In the first strategy, CRISPR/Cas9 is delivered along with a ssODN for the HDR-mediated correction of the SCD mutation (DeWitt, et al 2016, Pattabhi, et al 2019) or with an AAV encoding the entire *HBB* complementary DNA that serves as donor template for the site-specific replacement of the defective *HBB* gene (Dever, et al 2016). This universal approach is applicable to most patients' mutations and uses the endogenous promoter to drive expression of the transgene (unlike vector-based gene therapy approaches). In comparison to these HDR-dependent strategies that are currently limited by low efficiency in long-term repopulating stem cells, CRISPR/Cas9-mediated HbF reactivation is more efficient in HSPCs since it is achieved by the prevalent NHEJ pathway. Proof of concept experiments have established that HbF reactivation can be accomplished either by inactivation of the gene encoding the HbF transcriptional repressor protein BCL11A, specifically by mutating an erythroid-enhancer motif located in the gene's intronic sequence (Chang, et al 2017, Wu, et al 2019), or by disruption of its binding site located in the promoters of the gamma globin genes (Lux, et al 2019, Traxler, et al 2016, Ye, et al 2016). In the majority of studies, the humanised mouse transplantation model (reviewed in (Radtke, et al 2019)) was used to verify adequate engraftment and differentiation of engineered HSPCs. This model, however, is limited by the short-term monitoring of engraftment (< 6 months) and by the inability to measure haemoglobin output since peripheral erythroid differentiation is not supported in these

animals. A recent mouse model generated by engineering a homozygous Kit^{W41} allele was found to be more permissive to the production of human erythroid cells, but these are exclusively located in the bone marrow and are absent from peripheral blood (McIntosh, et al 2015). To circumvent these challenges, our group used the non-human primate transplantation model to document long-term (>2 years) engraftment of HSPCs edited by the NHEJ pathway to disrupt the BCL11A binding site, and peripheral blood HbF reactivation that correlated with editing achieved *in vivo* in nucleated cells (Humbert, et al 2019b). Together, these preclinical studies have enabled the initiation of several clinical trials that we discuss in the next section.

Genome editing is also actively being investigated as a novel treatment against human immunodeficiency virus (HIV). The description of a patient, known as the 'Berlin Patient', who was functionally cured of HIV following transplantation of allogeneic stem cells from a donor with a homozygous *CCR5* allele containing a 32-bp deletion (*CCR5Δ32*) (Hutter, et al 2009), was a strong impetus for the investigation of novel HSPC editing strategies. More recently, HIV remission was reported in a second case known as the 'London Patient' who received a similar transplantation with *CCR5Δ32* HSPCs but with a milder conditioning regimen (Gupta, et al 2019). CCR5 acts as a major coreceptor for HIV infection, and the generation of edited, CCR5-null, haematopoietic cells is expected to confer resistance to viral entry. Although initial work has aimed at specifically editing CD4+ T cells, being the major cell type targeted by the HIV virus, focus has recently switched toward the modification of HPSCs for a more durable response and a protection that also includes myeloid cells. Several studies have provided proof of concept results that NHEJ-mediated CRISPR/Cas9 inactivation of CCR5 in HSPCs does not impair engraftment in a mouse transplantation model and confers partial HIV resistance (Xu, et al 2017). These pre-clinical data served as a launching platform for testing in human patients as described in the next paragraph. While it is not entirely clear how much editing in nucleated cells will be required to achieve a complete cure, it is predicted that HIV infection will cause a gradual expansion in the number of edited and resistant cells (Perez, et al 2008, Peterson, et al 2018), due to their relative survival benefit, ultimately limiting the infection burden. Furthermore, this *CCR5*-editing approach may be combined with other editing strategies aimed at excising the integrated HIV proviral DNA from the host genome to eliminate the latent reservoir (Dash, et al 2019).

CRISPR/Cas9-editing has also been used as a powerful tool in cancer research, particularly for the rapid development of cancer immunotherapy. As we describe in the next paragraph, the vast majority of immunotherapy studies have focused on the modification of T-lymphocytes, for example, to express a chimeric antigen receptor (CAR) specific for a tumour antigen, but there are also some applications involving HSPC modification instead of T cells. CARs have been expressed in HSPCs to circumvent some of the limitations of CAR T cells, such as the short duration of the response or the exhaustion of the modified T cells (Larson and De Oliveira 2014). This strategy has so far employed retroviral vectors for the expression of the CAR, but current clinical CAR-T studies are using CRISPR/Cas9-editing to optimise CAR-T efficacy, and preclinical investigations into the use of CRISPR/Cas9-editing for CAR expression itself are ongoing.

Another approach using HSPCs aims to artificially create cancer-specific antigens by removing this same antigen from healthy cells to avoid toxicity. For example, specific treatment for myeloid malignancies, including acute myeloid leukaemia (AML), often focuses on CD33, a sialic acid binding receptor displayed on leukaemic cells but also on normal myeloid progenitors. For this reason, application of CD33-directed drugs such as the antibody-drug conjugate gemtuzumab ozogamicin (GO) results in significant on-target/off-leukaemia effects due to toxicity caused to healthy haematopoietic cells. Since allogeneic stem cell transplantation is already used to treat AML, we and others have demonstrated that CRISPR/Cas9 can safely remove CD33 from HSPCs and their derived progeny without altering engraftment capacity or biological function of these cells (Borot, et al 2019, Humbert, et al 2019a, Kim, et al 2018). Importantly, these studies demonstrated that haematopoietic cells generated from the edited and engrafted HSPCs were protected from the killing activity of GO, CD33/CD3 bispecific antibodies, or CD33 CAR-T cells, thus improving the outcome of AML therapies. This gene editing strategy also is likely to be applicable to other AML antigens such as CD123 or CD244 (Haubner, et al 2019) and to other forms of malignancies.

Beyond the diseases described here, HSPC editing has been applied to the treatment of other monogenic disorders such as Fanconi anemia, a hereditary defect of DNA repair in which causative mutations can be corrected by NHEJ-mediated repair of CRISPR/Cas9-induced DSBs (Roman-Rodriguez, et al 2019), or by using ZFN and the HDR pathway (Diez, et al 2017). X-linked severe combined immunodeficiency (SCID-X1) was also treated with a CRISPR/AAV-based strategy for targeted integration of a cDNA to functionally correct patient-derived HSPCs with as much as 45% efficiency (Pavel-Dinu, et al 2019). Similar to that described above for haemoglobinopathies, this strategy provides universal correction of all haematopoietic cell lineages by targeting the HSPC population, and allows for expression of the corrected gene under the control of the endogenous promoter. These are some examples for the most advanced functions using HSPC-directed CRISPR/Cas9-editing, but the repertoire of applications is expected to expand exponentially in the coming years.

T-cell based CRISPR/Cas9 therapeutic strategies

Beyond HSPCs, T cells have also been the target for genome editing mainly for the treatment of malignant conditions, by way of optimizing CAR-T cell efficacy, redirecting the T cell receptor (TCR), or by removing immune checkpoint inhibitors. Beyond the general advantages of genome editing over vector-based gene transfer that we highlighted earlier, it has become apparent that the site of CAR integration highly influences anti-tumour potency of the CAR-T cells, and this can be directed towards sites offering maximal efficacy with the use of genome editing strategies. CARs are engineered receptors consisting of an extracellular domain that recognises the antigen and an intracellular signal-activating domain. As an attempt to improve CAR-T cell performance, the Sadelain group used CRISPR/Cas9 and the HDR repair pathway to generate a uniform population of CD19-specific CAR T cells, in which the CAR transgene was precisely inserted at the TCR alpha locus (TRAC) (Eyquem, et al 2017). These T-cells showed CAR expression under the control of the native TCR promoter, and exhibited reduced exhaustion and increased activity in a mouse model of acute lymphoblastic leukaemia. Similarly, CRISPR/Cas9-mediated

deletion and knockout of the endogenous TCR has been employed to facilitate subsequent TCR redirection by delivery of a specifically targeted TCR addition by retroviral vector. T cells modified with this approach exhibited greater tumour antigen sensitivity in a mouse model as compared to cells modified by standard TCR gene transfer, because the native TCR was no longer competing with the newly inserted receptor (Legut, et al 2018).

Beyond the use of autologous T cells that have to be tailored to individual patients, and that are costly and time consuming to produce at therapeutic scale, there is significant benefit to the availability of universal allogeneic T cells that can serve as “off-the-shelf” ready-to-use agents (reviewed in (Graham, et al 2018)). The ability to multiplex CRISPR/Cas9 gene editing through the usage of several sgRNAs simultaneously in the same cell has been harnessed to overcome immunological incompatibility by inactivation of the endogenous TCR and beta 2 microglobulin gene (*B2M*, a component of MHC class I molecules, Figure 3) (Ren, et al 2017). Further investigation will determine whether expression of HLA-E in these cells is required to avoid NK cell-mediated lysis (Gornalusse, et al 2017). In turn, these cells can be modified further to express a CAR for anti-tumour activity and enhanced by the simultaneous knockout of immune checkpoint inhibitors such as programmed cell death protein (PD-1) (Ren, et al 2017). T cells can also be further engineered by inactivating CD52, a protein targeted by the monoclonal antibody alemtuzumab, to promote engraftment of modified cells through lymphodepletion/ immunosuppression (Poirot, et al 2015). Despite the great potential of engineering T cells by multiplex gene editing, caution must be taken to avoid the generation of chromosomal translocations and off-target mutations that can increase risk for oncogenic transformation. As we describe in the next section, these different flavours of engineered T cells are currently being tested in patients with various malignancies.

CRISPR/Cas as diagnostic and discovery tool for new targets in haematological malignancies

CRISPR/Cas9 editing is also a valuable tool in the discovery and validation of new therapeutic targets in haematological malignancies such as leukaemia, lymphoma and myeloma. Genome-wide CRISPR/Cas9 screens can be undertaken in malignant cell lines, to identify points at which disruption modulates growth or survival of the malignant clone, and have been described in detail elsewhere (Luo 2016). Postulated genes can be specifically interrogated in subsequent experiments to identify potential therapeutic targets such as FOXP1 in diffuse large B-cell lymphoma, DOT1L in multiple myeloma, MCL-1 in mantle cell lymphoma or several molecular effectors required for the regulation of the checkpoint molecule PD-1 in anaplastic lymphoma kinase-positive anaplastic large-cell lymphoma (Dafflon, et al 2020, Dengler, et al 2020, Felce, et al 2020, Zhang, et al 2019).

Modulators of cancer sensitivity to standard therapies can also be investigated, to provide insights into sensitivity and resistance mechanisms. For example, a CRISPR library screen in a Hodgkin lymphoma cell line has revealed two ubiquitin-editing enzymes as key regulators of sensitivity to brentuximab-vedotin (Wei, et al 2020).

CRISPR/Cas9 knockout provides more durable depletion than short interfering RNA applications, which have been previously used in such settings. This permits longer-term tracking of the effects of targeted knockouts, including *in vivo* effects following transplantation of edited cancer cell lines into mice. In situations where DSB-mediated gene knockout are not adequate, other options include loss-of-function through transcriptional repression (CRISPRi) or gain-of-function through transcriptional activation (CRISPRa) (Gilbert, et al 2014). In case of CRISPRi, a catalytically dead Cas9 (dCas9) protein is fused to a repressor domain such as Krüppel-associated box (KRAB) protein to silence genes by epigenetic mechanisms. In contrast, CRISPRa consists of a dCas9 fused to one or several activator domains such as the general control protein (GCN4) or virus protein 16 (VP16).

The CRISPR/Cas platform has also been harnessed as a sensitive tool for the detection of nucleic acid at very low concentration and with single-nucleotide specificity. This technology, named SHERLOCK (Gootenberg, et al 2017) (Specific High-Sensitivity Enzymatic Reporter UnLOCKing) is based on the promiscuous ribonuclease activity documented in some Cas proteins (not Cas9) upon DNA target recognition, which serves to activate a reporter and emit a signal upon detection of the target. This diagnostic tool has obvious applications in infectious diseases for the detection and quantification of viral and bacterial pathogens, but it has also proven useful in the detection of cancer-associated mutations from circulating cell-free DNA such as in non-small cell lung cancer (Gootenberg, et al 2018) or mutations in the *EGFR* or *BRAF* genes with allelic fractions as low as 0.1% (Gootenberg, et al 2017). We can even envision disease contexts where SHERLOCK will be used both for diagnosis and therapy using the Cas RNA editing/knockdown capability of this system.

Testing of CRISPR/Cas9-based therapies in clinical trials

Over 40 interventional trials of genome editing are currently registered with the United States (Clinicaltrials.gov) or European Union (EU Clinical Trials Register, EudraCT) clinical trials registries, targeting a multitude of benign or malignant and inherited or acquired conditions (summarized in Table I). The first trials involving *ex vivo* editing of T lymphocytes or HSPCs commenced in 2009, while trials investigating the direct *in vivo* delivery of editing agents were introduced later, since 2016. ZFNs and TALENs were used initially as the editing platform, but CRISPR/Cas9 has recently become the most common technology employed. For completeness, we describe clinical trials utilising all editing platforms and specify the type of nuclease used for each study (Table I).

Ex vivo editing of autologous HSPCs sourced from bone marrow or mobilised peripheral blood are the subjects of NHEJ editing for the treatment of β-haemoglobinopathies and HIV. Targeting of the *BCL11A* enhancer region with the aim of reversing the fetal-to-adult haemoglobin switch and increasing HbF levels is being investigated in adults for safety, tolerability and efficacy by CRISPR therapeutics and Vertex Pharmaceuticals (CTX001), and by Sanofi and Sangamo Therapeutics (BIVV003, ST400) as a treatment for both sickle cell disease and transfusion-dependent β-thalassaemia. Early results from the CTX001 study suggest successful amelioration of disease in first patients, but longer-term follow-up is required for more definitive results.

ZFNs were used for the generation of *CCR5*-knockout autologous CD4+ T cells, which were subsequently re-administered to HIV-positive patients in multiple clinical trials (Sangamo Therapeutics, University of Pennsylvania and National Institute of Allergy and Infectious Diseases). Interestingly, one study reported a significant increase in circulating T cells post-treatment, consistent with protection from HIV infection (Tebas, et al 2014). Medium-term persistence of *CCR5*-modified cells post-infusion was reported in this study, and, since then, other investigators have considered the use of repeated administration of similar cells to provide longer-term efficacy (NCT02225665, Sangamo Therapeutics). The application of HSPCs as an alternate editing target has recently become a more favoured approach to the maintenance of a long-lasting HIV-resistant population. Disruption of the *CCR5* locus in autologous or allogeneic HSPCs prior to transplantation has therefore been studied as a method to confer HIV resistance to the mature progeny of these modified stem cells. Results from trial NCT03164135 were recently reported for one HIV patient suffering from acute lymphoblastic leukaemia who received donor stem cells from an HLA-compatible donor (Xu, et al 2019). *CCR5*-edited HSPCs stably engrafted, as represented by 5 to 8% editing frequencies detected in bone marrow and blood at 19 months post-transplant, spanning multiple lineages. Importantly, no adverse effects or off-target activity related to gene editing was reported. While the efficiency of post-transplantation editing in this patient was not adequate to achieve a cure for HIV infection, these data confirm that *CCR5*-edited cells can stably engraft and persist long-term without complication.

The most advanced clinical trials testing gene edited T cells as cancer immunotherapeutic agents used TALENs to knock out the endogenous TCR and CD52 genes, and a lentiviral vector that expressed CD19-directed CAR to treat B-cell acute lymphoblastic leukaemia (Qasim, et al 2017). This initial study lead to the launch of two further clinical trials investigating the use of the CD19-directed CAR-T cells in both children (NCT02808442) and adults (NCT02746952). In 2018 two separate CD19-directed autologous CAR-T products (tisagenlecleucel or “Kymriah” from Novartis and axicabtagene ciloleucel or “Yescarta” from Kite Pharma) were granted a license by the EMA and FDA to treat aggressive B-cell malignancies in specific patient populations. The CD19 CAR Lisocabtagene maraleucel developed and tested by Juno (now Bristol-Myers Squibb) for the treatment of adult patients with relapsed or refractory large B-cell lymphoma (LBCL) is currently undergoing priority review by FDA (Abramson, et al 2019).

A similar editing approach is also being tested by Cellectis for AML (NCT03190278) and blastic plasmacytoid dendritic cell neoplasms (NCT03203369) using CD123-modified CAR-T cells. Clinical trials investigating CAR-T cell engineering currently involve lentiviral transduction of the CAR transgene, and adjuvant strategies based on gene editing are expected to improve CAR-T specificity, efficacy and longevity. Examples include increasing activity by *PD-1* or *HPK1* gene knock-out, or disruption of *CD52* to confer alemtuzumab resistance on the modified CAR-T cells.

PD-1 checkpoint inhibitor expression has been disrupted *ex vivo* in autologous T cells prior to expansion and reinfusion, with the aim of increasing the anti-cancer potency of edited cells, a strategy recently trialed for the treatment of oesophageal (NCT02800369, NCT03081715), non-small cell lung and EBV-driven malignancies (NCT02793856,

NCT03044743). CRISPR/Cas9 knock-out of the endogenous TCR has been employed to facilitate subsequent T-cell redirection by delivering specifically targeted TCR additions. Cancers targeted in trials of such redirected T cells include multiple myeloma and numerous solid tumours (NCT03399448). Results from three patients treated in this trial demonstrated safety and feasibility of engineering T cells with multiplex CRISPR/Cas9 to remove both the endogenous TCR and the checkpoint molecule PD-1, and with a lentiviral vector expressing a specific TCR to redirect these cells to target the cancer-testis antigens NY-ESO-1 and LAGE-1 (Stadtmauer, et al 2020). As was hypothesised, edited T cells showed a much more durable response as compared to results from other trials in which T cells retaining endogenous TCR and PD-1 expression were employed. However, the efficacy of these engineered T cells was ambiguous as clinical progression occurred post treatment in all patients. Residual tumour present after T cell infusion showed a reduction in target antigen expression, demonstrating a possible mechanism for tumour evasion. While minimal off-target CRISPR/Cas9 activity was documented in engineered cells, chromosomal translocations were observed at a frequency of less than 1% (discussed further below).

Considerable efforts are being made to improve the accessibility and reduce the cost of these gene editing therapies. This can be accomplished in principle by direct administration of the editing reagents to patients, bypassing the need to modify cells *ex vivo*. Intravenous delivery of AAV-packaged ZFN editing reagents to enlist the HDR repair pathway and deliver a functional copy of the therapeutic gene at the albumin locus is currently being investigated by Sangamo Therapeutics for the treatment of several genetic disorders. These include treatment of severe haemophilia B by delivery of the factor IX gene (NCT02695160) and mucopolysaccharidosis types I and II by targeted insertion of a corrected α-L-iduronidase (NCT02702115) or iduronate 2-Sulfatase gene (NCT03041324), respectively, into hepatocytes. Direct delivery of CRISPR/Cas9 editing reagents into the eye is also being studied for the control of visual loss caused by one subtype of Leber congenital amaurosis. This study, known as EDIT-101 (Editas Medicine and Allergan), is designed to remove an aberrant splicing site caused by a point mutation in the *CEP290* gene, which results in production of a dysfunctional CEP290 protein. The AAV5 vector employed in EDIT-101 contains two guide RNA molecules and the Cas9 protein, and aims to remove the aberrant splice site created by the mutation by using the NHEJ pathway (Maeder, et al 2019). Treatment of the first patient in that study was announced in March 2020.

While many trials are still in early phase 1 stages, others include progression to phase 2 studies, and therefore robust data on therapeutic efficacy as well as safety is expected to emerge. It is encouraging to note that many investigators recognise the importance of long-term follow-up, with ongoing monitoring of up to 15 years integrated into the treatment protocol of some studies and a separate long-term follow-up study now registered for β-haemoglobinopathy patients who have received CTX001 on trial (NCT04208529).

Challenges and limitations to therapeutic CRISPR/Cas9 genome editing

Significant challenges to the clinical application of CRISPR/Cas9 therapies still remain to be overcome. These include genotoxicity caused both by the editing procedure and cytotoxic conditioning regimens often administered alongside; subtherapeutic levels of edited cells

persisting post-treatment; and notable practical limitations such as cost and feasibility issues. It remains to be seen how effectively these will be tackled in current clinical trials, and it will be some time before data are mature enough to answer questions regarding long-term efficacy and toxicities.

Potential harms of therapeutic genome editing

There are still issues of safety as well as efficacy that must be addressed before routine clinical application of CRISPR/Cas9 editing strategies can be recommended (Demirci, et al 2018). Potential harms include off-target DNA cleavage at sites similar to the intended nuclease target, and chromosomal rearrangement events. Off-target activity may, for example, result in silencing of tumour suppressor genes or introduce gain-of-function mutations affecting oncogenes (Baylis 2018, Romero, et al 2019, Schaefer, et al 2017). Genomic rearrangements such as translocations and large deletions may result from on- or off-target DSBs and may have similar effects (Bak, et al 2018b). Even though these events are expected to occur at very low frequency, they will be a major concern for clinical translation if they induce oncogenic transformation of targeted cells (Gonzalez-Romero, et al 2019, Jeong, et al 2019). This is particularly a concern where a multiplexing CRISPR/Cas9 editing approach is applied. Autologous T cells engineered at multiple loci for the treatment of advanced myeloma or sarcoma showed multiple translocations events that were not only detectable in the infusion product but also persisted *in vivo* for up to 170 days after infusion in all three treated patients. Some reassurance was inferred from a decline in frequency of these events over time suggesting that they did not confer a selective growth advantage, but the outcome may be different with alternative multiplex targets or off-target DSBs (Stadtmauer, et al 2020).

These risks are especially concerning where HSPCs are the intended target of genome editing, given their high proliferation and differentiation capacity (Cornu, et al 2017, Gonzalez-Romero, et al 2019). While oncogenic risk is predicted to be significantly lower than with gene transfer therapy delivered by integrating lentiviral vectors (Braun, et al 2014, Howe, et al 2008), this possibility remains a realistic concern. Given that many of the currently active genome editing trials target non-malignant conditions such as haemoglobinopathies, even a small increase in risk of malignancy must be weighed carefully. The development of better tools to predict such adverse genetic events, and of more sensitive and standardised assays to detect them, still requires more work (Cornu, et al 2017). Unbiased, genome-wide tools allowing the long-term biological consequences of such events to be predicted are also needed (Tsai and Joung 2016).

Protocols for *ex vivo* modification of HSPCs followed by autologous re-transplantation still require the application of cytotoxic conditioning regimens. These are associated with acute complications such as myelosuppression and organ dysfunction, and long-term risk of secondary malignancies (Baker, et al 2003, Lidonnici and Ferrari 2018), thus amplifying risks for oncogenesis in such treatment protocols. An important challenge for clinical translation of gene therapy or genome editing approaches will be to use reduced-intensity conditioning regimens to 19tilizin toxicity without compromising engraftment, such as non-genotoxic antibody-drug conjugates (ADCs).

As investigators increasingly focus on *in vivo* administration of CRISPR reagents, the potential for unintended editing of the germline must be ethically considered. The possibility that gametes may be affected during *in vivo* genome editing is being investigated in current trials, although the consequences of such editing on any future progeny are largely unknown. Following the recent CRISPR/Cas9 editing of human embryos that were subsequently implanted and allowed to progress to delivery, the genome editing community and regulatory bodies have united in condemning any form of editing that would directly affect the germline. Substantially more research is needed to determine potential side-effects of germline editing including ethical and societal implications before this could be acceptable (Cyranoski and Ledford 2018).

Together, there must be careful consideration of the risk versus benefit balance of each CRISPR/Cas9 editing therapy for each patient. The risks of the disease itself and the toxicities of other potential treatments must be weighed against the known and unknown disadvantages associated with genome editing therapies.

Subtherapeutic levels of edited cells in vivo

Achieving and sustaining adequate numbers of edited cells *in vivo* to confer therapeutic benefit remains a major limitation to the clinical application of this technology to many diseases. This is particularly pertinent where HDR editing is applied to HSPCs, where a rapid fall-off is observed after transplantation and engraftment in many preclinical studies, even when respectable editing efficiency was initially obtained in infused cells (Dever, et al 2016, Genovese, et al 2014, Gomez-Ospina, et al 2019, Pattabhi, et al 2019). In comparison, HDR rates reported in T cells to insert a CAR within the TRAC locus were more encouraging. Very recently, αβ T cells treated with CRISPR/Cas9 editing and an AAV donor template resulted in >75% expression of the CAR along with >90% TCR loss (Wiebking, et al 2020). These unprecedented rates of targeted integration of large gene cassettes bode well for improved HDR efficiencies in other T-cell settings but additional optimization will be required before such impressive results are seen in long term engrafting stem cells. Although the clinical trial of CRISPR/Cas9 editing of HSPCs for ablating CCR5 expression as a treatment for HIV showed promising results in one patient (NCT03164135, (Xu, et al 2019)), the numbers of modified cells achieved post-transplant were sub-therapeutic, illustrating the ongoing challenges in this area. Stem cells with long term repopulating potential are one of the most challenging cell types to modify in this regard, due at least in part to their quiescent nature and therefore minimally active intracellular HDR gene-repair pathways. Apoptosis or cell cycle arrest mediated by p53 induction occurs in the majority of edited HSPCs, providing, at least in part, an explanation for their resistance to editing and high rates of cell death (Haapaniemi, et al 2018, Ihry, et al 2018). HDR-editing with an added AAV donor template resulted in a cumulative effect on p53 pathway activation but, interestingly, the defect seen in colony forming potential and engraftment of treated cells could be improved by transient p53 inhibition (Schiroli, et al 2019).

Pre-existing immunity to genome editing agents or delivery vectors and immunogenicity of the treatment itself has the potential to render *in vivo* treatment ineffective or trigger adverse reactions. Pre-existing anti-Cas9 antibodies and antigen-specific T cells are present in a

significant proportion of the population, presumably stimulated by prior exposure to Cas9-producing bacteria (Charlesworth, et al 2019, Simhadri, et al 2018). The development of adverse immune responses to novel antigens presented by CRISPR/Cas9-edited cells must thus also be considered (Baylis 2018, Kang, et al 2018).

Practical limitations

Beyond the issues of efficacy and genetic risk, there are notable practical challenges involved in the delivery of CRISPR editing therapies to patients who may benefit from them. Access to the target cells for editing, costs, and feasibility of delivery are also potentially constraining factors. Accessing adequate numbers of HSPCs for *ex vivo* modification is not straightforward. Cells must either be harvested directly from the bone marrow in an invasive and painful procedure (Ho 2019, Siddiq, et al 2009), or mobilised into the peripheral blood prior to collection by apheresis. While the latter is less invasive, failure to collect adequate HSPCs is not uncommon (Duong, et al 2014), and mobilization is particularly difficult in patients with sickle cell disease where G-CSF administration risks precipitating life-threatening vaso-occlusive crises (Fitzhugh, et al 2009). Achieving access to tissue stem cells for CRISPR editing, such as in muscular dystrophies or cystic fibrosis, is even more challenging (Azvolinsky 2019).

The expenses involved in developing and delivering CRISPR genome editing therapies impose further limitations on this field. There is a degree of mismatch between the long-term investment required for the successful development of genome editing treatments and the appetite for immediate results and short-term rewards often demonstrated by industry investors (Sheridan 2018). Even post-development, the high costs of the genome editing procedure, and the necessary clinical interventions required to deliver such therapies, are likely to make access to this new genre of next-generation treatment unattainable for the vast majority of patients who may otherwise stand to benefit from them (Wilson and Carroll 2019).

In developed countries there must be open dialogue between patient and carer groups, health care leaders and the public, regarding the resource allocation implications of bringing genome editing therapies into the clinic. In the developing world there are even more hurdles to be overcome. Cell processing and manufacture of genome editing products must be to Good Manufacturing Practice (GMP) standards and in approved institutions. The clinical facilities required to deliver such treatments are also unavailable to most of the global population. The majority of patients with sickle cell disease, for example, are situated in Sub-Saharan Africa, where only a tiny minority have access to a modified autologous haematopoietic stem cell transplant procedure. Research efforts must focus on ensuring that genome editing therapies are deliverable to the patients who need them most, to avoid further exacerbating health inequalities between those of different socioeconomic circumstances (Baylis 2018, Wilson and Carroll 2019).

Finally, regulation of this rapidly progressive clinical field is a major challenge facing regulatory authorities and governments, as the speed of scientific progress, including broadening of the potential applications for such technology, outstrips the ability of many

such bodies to keep pace. Genome editing therapies are being investigated for a multitude of diseases and cell types, utilising numerous editing strategies. A ‘one size fits all’ approach to the assessment of efficacy or safety of these applications is not realistic nor, therefore, to their regulation. Follow-up post-treatment is a particularly important area to be addressed. Lifelong follow-up to detect adverse events would be ideal; however, in many cases the resource and logistical implications would be prohibitive, and there is currently no consensus amongst governing bodies as to the required length of surveillance (Abou-El-Enein, et al 2017). Laws and regulations will inevitably reflect ongoing debate regarding social and ethical consequences of genome editing as well as biological effect, adding further complexity to this area (Abou-El-Enein, et al 2017, Baylis and McLeod 2017).

The future of CRISPR genome editing in the clinic

Upcoming developments predicted to facilitate further expansion and success of CRISPR/Cas9 editing therapies include methods by which levels of edited cells may be increased *in vivo* to more reliably allow for therapeutic benefit and strategies to further improve the safety profile of such treatments. *In vivo* delivery of therapeutic CRISPR reagents is likely to overtake *ex vivo* cellular modification strategies in the future, at least for some applications, and is already in use for the treatment of Leber congenital amaurosis type 10 (Table I), but this modality is not yet being applied to HSPC or T-lymphocyte modification. The potential applications for CRISPR editing are already expanding rapidly, and as the safety and efficacy of these technologies improves, this growth is expected to continue.

Improving in vivo editing efficiency

The challenge of achieving therapeutic levels of edited cells post-transplantation may be tackled in various ways. Other than increasing editing efficiency at the point of initial application, providing an engraftment or survival advantage to edited cells or enabling positive selection of cells harbouring the desired genetic modification would be expected to increase the proportion of modified cells post treatment. *In vivo* selection for modified cells may happen naturally for some disorders such as SCID-X1 or Fanconi Anemia where edited/corrected cells have a survival advantages over uncorrected cells, or it may have to be engineered, for example to increase resistance to cytotoxic drugs (Nagree, et al 2015).

In vivo positive selection of CRISPR/Cas9 edited cells is an attractive possibility, and one that is already under investigation in pre-clinical studies. An example of such an approach is the introduction of a chemoresistance gene cassette to edited cells, conferring resistance to a subsequently-applied chemotherapeutic agent and thereby resulting in positive selection pressure that favours the edited cells (Beard, et al 2010, Falahati, et al 2012, Paul, et al 2018, Wang, et al 2019). While further study is required to develop the use of non-genotoxic selection agents, these reports provide proof of concept that such an approach may allow for *in vivo* enhancement of subtherapeutic editing levels.

Other positive selection strategies to increase the proportion of CRISPR/Cas9 edited cells while still *ex vivo* are under investigation, by conferring a survival advantage to modified cells or via an alternative selection method such as flow cytometric purification of marked

edited cells (Agudelo, et al 2017, Dever, et al 2016). Another method to improve *in vivo* editing levels where HSPCs are the targets of modification is to improve engraftment and survival capacity of edited cells post transplantation, thereby giving them a competitive advantage. One example of this strategy is the enhancement of CXCR4 expression on edited HSPCs, which has been shown to improve homing to and engraftment in the bone marrow niche (Arai, et al 2018, Brenner, et al 2004, Kahn, et al 2004).

Due to the requirement of CRISPR/Cas9 systems for a short sequence motif adjacent to target sites known as protospacer adjacent motif (PAM), a significant portion of the genome has not been amenable to editing. Recent efforts focused on the generation of “PAMless” Cas9 variants, which widely expanded the therapeutic applicability of this nuclease platform to chromosomal regions that were previously inaccessible to editing (Walton, et al 2020). These variants were generally found to have comparable activity to the original Cas9 enzyme in human cell lines but specificity remains to be evaluated in T cells and HSPCs.

Safety

There are many proposed methods for reducing genotoxic risk in CRISPR/Cas9 editing. The most established strategies under investigation are the use of bioinformatic prediction and design tools to produce editing compounds less likely to induce on- or off-target adverse effects (reviewed in (Liu, et al 2020)) and higher fidelity Cas9 preparations to reduce off-target mutagenesis (Chen, et al 2019, Kleinstiver, et al 2019, Vakulskas, et al 2018). Targeting integration of new genetic material by directing the HDR machinery to ‘safe harbour’ loci, where the risks of DNA DSBs or undesired INDELS would not affect functional DNA sequences, is another way by which the safety of CRISPR/Cas9 editing can be improved (Gomez-Ospina, et al 2019, Hong, et al 2017, Papapetrou, et al 2011, Yada, et al 2017). Anti-CRISPR/Cas proteins known as “Acr” could also be used as a tunable mechanism to limit off-target editing. Experiments conducted in CD34+ HSPCs showed feasibility of this approach when CRISPR/Cas9 was delivered as RNPs (Shin, et al 2017) or via adenoviral vectors (Li, et al 2018). It is encouraging to note that no off-target edits were reported in the liver of mice after *in vivo* delivery of CRISPR/Cas9 targeting the *PCSK9* gene (Akcakaya, et al 2018), demonstrating that this system can be highly specific for carefully tested sgRNA sequences, particularly for *in vivo* genome editing applications.

Novel editing platforms recently reported employing modified CRISPR/Cas9 coupled to deaminase domains to catalyze precise A-to-G or C-to-T changes in a target sequence without the requirement for dsDNA breaks. These so-called base editors allow for the substitution of a single nucleotide for an alternate one to correct a disease-causing mutation or to disable function of a gene through the introduction of a stop codon or the disruption of conserved splicing sites (Gaudelli, et al 2017, Komor, et al 2016). The versatility of possible genetic alterations introduced at a given target was also recently enhanced with a new platform known as prime editors, which consist of CRISPR/Cas9 linked to a reverse-transcriptase. Interestingly, these enzymes can introduce novel genetic material into chromosomal DNA without the need for DNA DSBs and bypassing the HDR pathway; this method decreases genotoxicity, although this risk is not entirely eliminated (Anzalone, et al 2019, Gaudelli, et al 2017, Komor, et al 2016). Importantly, base editors have already been

validated in both T cells (Webber, et al 2019) and in HSPCs (Zeng, et al 2020), even in the context of multiplex genome editing.

Finally, current protocols for modified autologous HSPC transplantation all require administration of genotoxic conditioning chemotherapy. A further improvement to the safety of such procedures will be the introduction of non-genotoxic targeted conditioning regimens such as ADCs. These antibodies can specifically target stem cells in the BM niche, and recent studies using anti-CD117 ADCs have demonstrated feasibility of this approach (Kwon, et al 2019, Li, et al 2019, Palchaudhuri, et al 2016, Srikanthan, et al 2020).

Improving cost and accessibility of CRISPR/Cas9 genome editing therapies

The majority of currently active CRISPR/Cas9 clinical trials rely on *ex vivo* editing procedures, but the first *in vivo* editing trials are already underway. Alternative methods of packaging and conveying CRISPR reagents, such as non-viral gold or lipid nanoparticle delivery, may improve options for *in vivo* delivery, and potentially also reduce the cytotoxicity and immunogenicity of such treatments (Kulkarni, et al 2018, Shahbazi, et al 2019).

In vivo delivery of CRISPR/Cas9 editing therapies have significant advantages in such that the *ex vivo* cellular processing and modification steps are avoided. This is likely to reduce both risk and cost for many patients, as the procedures associated with cell collection and re-transplantation are circumvented. *In vivo* delivery of CRISPR/Cas9 therapies is also expected to facilitate application in regions where GMP and transplantation facilities are unavailable.

Another method by which treatment costs may be reduced is by improving the accuracy with which true target cells are selected for editing. Where HSPCs are the target of CRISPR/Cas9 editing, it is possible to select a population of cells enriched for true, long-term repopulating haematopoietic stem cells based on the immunophenotype CD34+/CD90+/CD45RA-, reducing the number of cells submitted to CRISPR editing and, therefore, associated reagent costs by up to 90%, while still providing adequate long-term engraftment and differentiation post-modified transplant in preclinical studies (Humbert, et al 2019b, Radtke, et al 2017). A parallel strategy may be possible for other cell types.

In conclusion, the application of CRISPR/Cas9 editing to the cure of human disease is already delivering promising results, and its breadth and efficacy of usage is expected to continue advancing. While challenges facing the clinical application of genome editing therapies are recognised, many strategies to overcome each of these are already in development. It may in the end be practical issues of cost and delivery that are the last remaining limitations to the true potential of this technology to combat human disease.

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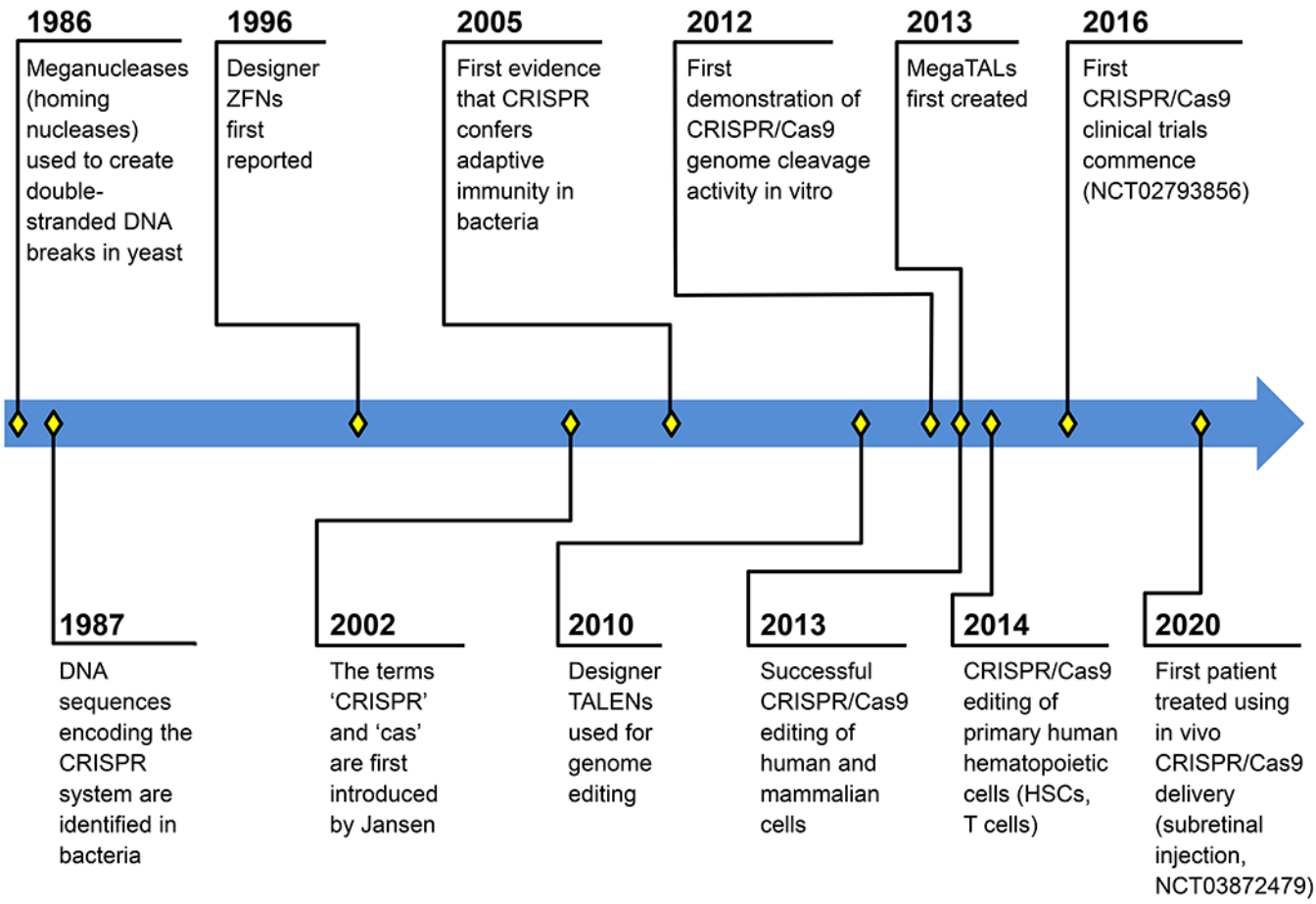

Figure 1. Timeline describing the important chapters of CRISPR/Cas9 development and other editing platform development.

Notable publications associated with time events: **1986:** (Kolodkin, et al 1986); **1987:** (Ishino, et al 1987); **1996:** (Kim, et al 1996); **2002:** (Jansen, et al 2002); **2005:** (Bolotin, et al 2005, Mojica, et al 2005, Pourcel, et al 2005); **2010:** (Christian, et al 2010, Li, et al 2011); **2013:** (Boissel, et al 2014, Cho, et al 2013, Cong, et al 2013, Mali, et al 2013a, Mali, et al 2013b); **2014:** (Hendel, et al 2015, Mandal, et al 2014).

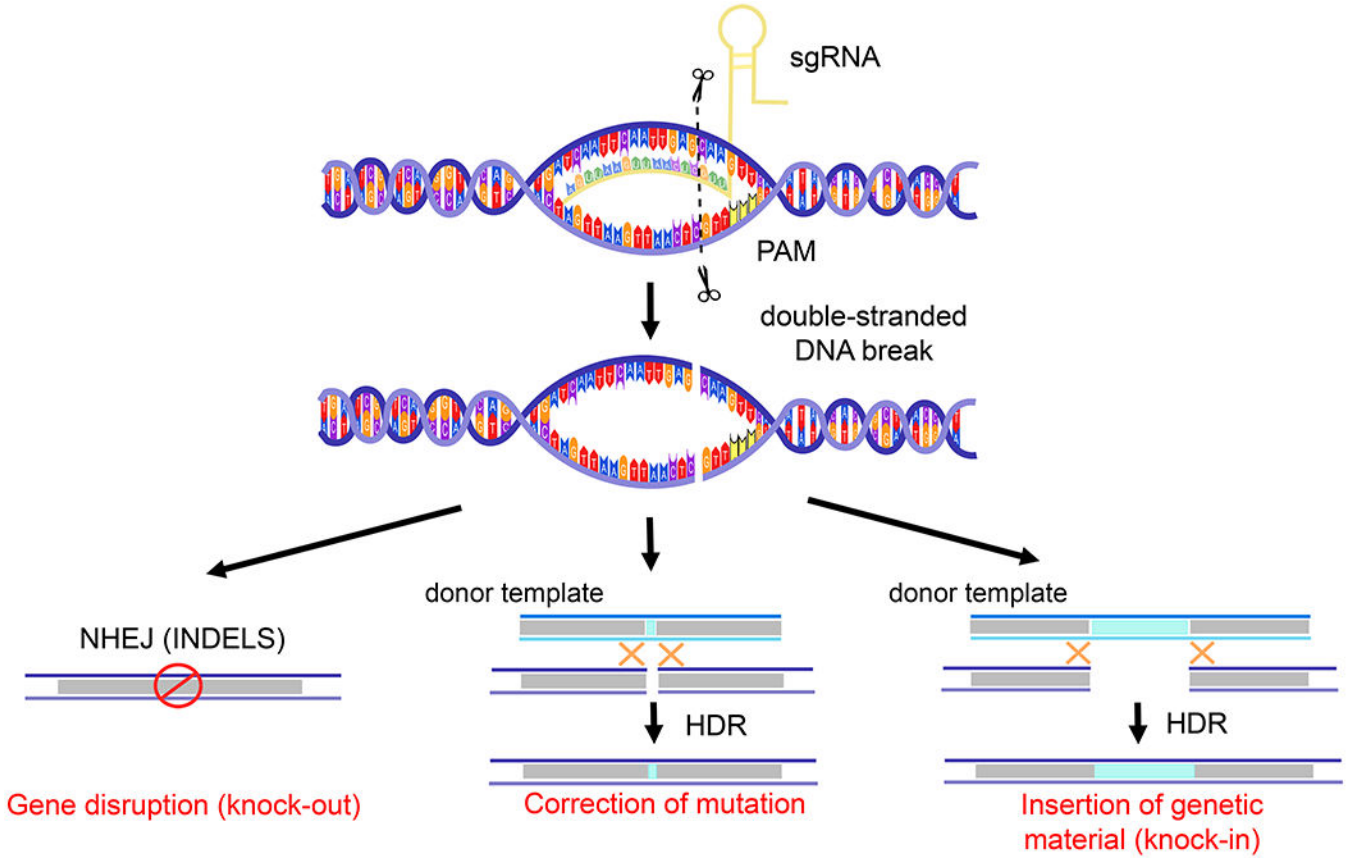

Figure 2. Simplified mechanism of CRISPR/Cas9 genome editing.

Single guide RNA (sgRNA)-mediated recognition of the chromosomal target sequence via the protospacer adjacent motif (PAM) sequence. A double-stranded cut occurs 3 nucleotides upstream of the PAM sequence and enlists the non-homologous end joining (NHEJ) or homology-directed repair (HDR) pathways. INDELS= insertions and deletions.

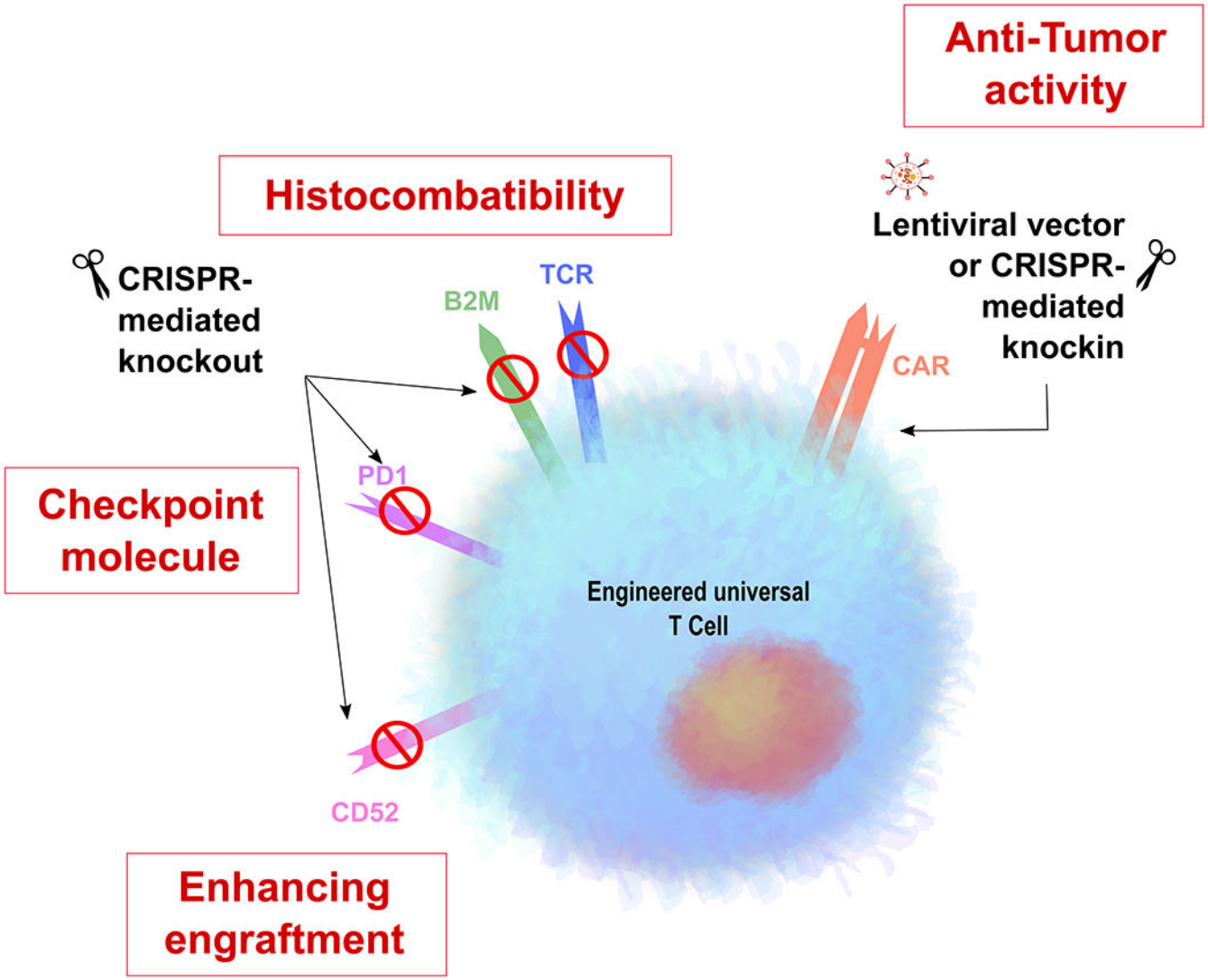

Figure 3. Schematic of engineering of universal T cells by CRISPR/Cas9-knockout of different receptor molecules or by knockin of a CAR using lentiviral vector or CRISPR/Cas9-knockin.
B2M= Beta 2 microglobulin; CD52=cluster of differentiation 52; CAR=chimeric antigen receptor; PD1= Programmed cell death protein 1; TCR= T cell receptor.

Table I.

Summary of currently registered clinical trials

Condition	Delivery and target cells	Genome editing platform(s) and target gene	Phase	Age	Dates (estimated study completion dates given where not complete)	Trial registration number (ClinicalTrials.gov or EU Clinical Trials Register)
Completed/status unknown						
HIV	*Ex vivo*: autologous T-cells	ZFN: NHEJ editing of *CCR5*	1, 1/2	Adult	Jan 2009-Jan 2013	NCT00842634
					Dec 2009-Dec 2014	NCT01044654
					Dec 2011-Jul 2017	NCT01543152
					Aug 2014-Jun 2018	NCT02225665
					Apr 2015-Mar 2019	NCT02388594
Oesophageal cancer	*Ex vivo*: autologous T-cells: PD-1 knockout	CRISPR/Cas9: NHEJ editing of *PD-1*	1	Adult	Mar 2017-Feb 2018	NCT03081715
Human papillomavirus (HPV) related; cervical Intraepithelial; neoplasia	*In vivo:* Topical gel / pessary targeting cervical epithelium	TALEN, CRISPR/Cas9, ZFN: NHEJ editing at HPV *E6 / E7*	1	Adult	Dec 2017-July 2017	NCT02800369
					Jan 2018-Jan 2019	NCT03057912
					Jan 2018-Jan 2019	NCT03226470
Blastic plasmacytoid dendritic cell neoplasm	*Ex vivo*: autologous CD123 CAR T-cells	TALEN: NHEJ deletion of TCR, disruption of *CD52* gene	1	Adult	Jun 2017-Jun 2019	NCT03203369
Currently active						
Sickle cell disease	*Ex vivo*: autologous HSPCs	CRISPR/Cas9, ZFN: NHEJ disruption of *BCL11A* enhancer	1/2	Adult	Nov 2018-May 2022	NCT03745287
					Jun 2019-Apr 2023	NCT03653247
Transfusion-dependent β-thalassaemia	*Ex vivo*: autologous HSPCs	CRISPR/Cas9, ZFN: NHEJ disruption of *BCL11A* enhancer	1/2	Adult	Mar 2018-Dec 2022	NCT03432364
					Sept 2018-May 2022	NCT03655678
Sickle cell disease and transfusion-dependent β-thalassaemia	(Long-term follow-up study in subjects who received CTX001 on studies NCT03655678 or NCT03745287)				Feb 2021-Sept 2039	NCT04208529

Condition	Delivery and target cells	Genome editing platform(s) and target gene	Phase	Age	Dates (estimated study completion dates given where not complete)	Trial registration number (ClinicalTrials.gov or EU Clinical Trials Register)
HIV	*Ex vivo*: autologous/ allogeneic HSPCs	CRISPR/Cas9, ZFN: NHEJ disruption of *CCR5*	1	Adult	Jul 2015-Apr 2022	NCT02500849
					May 2017-May 2021	NCT03164135
Non-small cell lung cancer; Epstein-Barr virus-associated malignancies	*Ex vivo*: autologous T cells	CRISPR/Cas9: NHEJ disruption of *PD-1*	1/2	Adult	Aug 2016-Jan 2020	NCT02793856
					Apr 2017-Mar 2022	NCT03044743
Multiple myeloma; malignant melanoma, synovial sarcoma, myxoid / round cell liposarcoma	*Ex vivo*: autologous T cells	CRISPR/Cas9: NHEJ disruption of endogenous *TCR* and *PD-1*	1	Adult	Sept 2018-Jan 2033	NCT03399448
B cell acute lymphoblastic leukaemia or lymphoma; non-Hodgkin lymphoma; T cell acute lymphoblastic leukaemia or lymphoma; T cell lymphoma; multiple myeloma; acute myeloid leukaemia; mesothelin-positive solid tumours; HIV	*Ex vivo*: autologous / allogeneic T-cells: CAR-T cells	CRISPR/Cas9, ZFN or TALEN: NHEJ disruption of multiple targets including endogenous *PD-1, HPK1, CD52*	1, 1/2	Paediatric, adult	Jun 2016-July 2020	NCT02808442
					Aug 2016-July 2020	NCT02746952
					Jun 2017-May 2022	NCT03166878
					Jun 2017-Jun 2021	NCT03190278
					Jan 2018-May 2022	NCT03398967
					Jun 2018-Jun 2020	NCT03545815
					Nov 2018-May 2020	NCT03747965
					Jul 2019-Dec 2025	NCT03617198
					Jul 2019-Aug 2026	NCT04035434
					Aug 2019-Aug 2024	NCT04037566
					Oct 2019-Oct 2021	NCT04150497
					Nov 2019-Nov 2022	NCT04142619
					Mar 2020-May 2038	NCT03690011
					May 2016-	2015-004293-15
					May 2016-	2016-000296-24
					Information unavailable	2019-003462-40
Multiple myeloma	*Ex vivo:* allogeneic T-cells:	CRISPR/Cas9	1	Adult	Jan 2020-Jan 2027	NCT04244656

Condition	Delivery and target cells	Genome editing platform(s) and target gene	Phase	Age	Dates (estimated study completion dates given where not complete)	Trial registration number (ClinicalTrials.gov or EU Clinical Trials Register)
Mucopolysaccharidosis (I, II)	*In vivo*: Intravenous infusion targeting hepatocytes	AAV-delivered ZFN: HDR insertion of *IDS* or *IDUA* genes	1/2	Paediatric, adult	May 2017-Jan 2022	NCT02702115
					May 2017-Feb 2022	NCT03041324
Severe haemophilia B	*In vivo*: Intravenous infusion targeting hepatocytes	AAV-delivered ZFN: HDR editing	1	Paediatric, adult	Nov 2016-Jan 2021	NCT02695160
Leber congenital amaurosis type 10	*In vivo*: Subretinal injection	AAV-delivered CRISPR/Cas9: NHEJ to restore activity of *CEP290* gene	1/2	Paediatric, adult	Sept 2019-Mar 2024	NCT03872479
Transfusion-dependent β-thalassaemia	*Ex vivo*: autologous HSPCs	CRISPR/Cas9: HDR correction of *HBB*	1	Paediatric, adult	Jan 2019-Jan 2021	NCT03728322