Molecular Therapy
Review

Genome editing in large animal models

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Although genome editing technologies have the potential to revolutionize the way we treat human diseases, barriers to successful clinical implementation remain. Increasingly, preclinical large animal models are being used to overcome these barriers. In particular, the immunogenicity and long-term safety of novel gene editing therapeutics must be evaluated rigorously. However, short-lived small animal models, such as mice and rats, cannot address secondary pathologies that may arise years after a gene editing treatment. Likewise, immunodeficient mouse models by definition lack the ability to quantify the host immune response to a novel transgene or gene-edited locus. Large animal models, including dogs, pigs, and non-human primates (NHPs), bear greater resemblance to human anatomy, immunology, and lifespan and can be studied over longer timescales with clinical dosing regimens that are more relevant to humans. These models allow for larger scale and repeated blood and tissue sampling, enabling greater depth of study and focus on rare cellular subsets. Here, we review current progress in the development and evaluation of novel genome editing therapies in large animal models, focusing on applications in human immunodeficiency virus 1 (HIV-1) infection, cancer, and genetic diseases including hemoglobinopathies, Duchenne muscular dystrophy (DMD), hypercholesterolemia, and inherited retinal diseases.

INTRODUCTION

Animal models are indispensable elements of translational research that have contributed enormously to our enhanced understanding of disease pathophysiology, transplantation biology, and gene therapy. For therapeutic development, animal models have been used to identify novel targets, evaluate dosing regimens, assess pharmacokinetic/pharmacodynamic (PK/PD) relationships, and, importantly, measure the therapeutic index of novel drugs. While small animal models (e.g., rodents) are generally more accessible and cost effective, they differ from humans in core biological processes such as hematopoiesis and immunological response[1,2] and have demonstrated limitations in generating clinically relevant data for human diseases including cancer,[3,4] neurodegenerative diseases,[5,6] diabetes,[7] and stroke.[8] Large animals, including canines, non-human primates (NHPs), and pigs, may help to bridge the gap between rodent models and clinical studies since they are more similar to humans in anatomy, physiology, and metabolism (Table 1).[9–14] Compared to rodents, large animals have a considerably longer lifespan and thus enable multi-year studies to evaluate the long-term safety and efficacy of gene editing therapeutics, an especially critical aspect, for example, when assessing the impacts of off-target gene editing.[15] Furthermore, given that large animals are closer in size to humans than rodents, the dosage and clinical feasibility of large-scale manufacturing can be assessed more directly. This is a critical and often overlooked aspect of translational research, and many manufacturing protocols for generating gene editing reagents or gene-modified cell products for small animals are often not readily scalable for applications in large mammals. The field of transplantation biology was pioneered in canine models,[16] which also closely recapitulate many human malignancies, cardiovascular disease, and neuromuscular disorders.[17–19] Early gene therapy and transplantation studies were conducted in dog and NHP models, since mouse models failed to identify important barriers to successful clinical implementation, such as human leukocyte antigen (HLA) matching and effective conditioning regimens.[9,20] As a result, large animal studies helped develop clinically translatable protocols for generating gene-modified cells and achieving efficient *in vivo* engraftment. Here we review the development and preclinical evaluation of therapeutic genome editing strategies in large animal models, with a focus on applications in human immunodeficiency virus (HIV), cancer, blood disorders, muscular dystrophy, and retinal degenerations.

OVERVIEW OF GENE EDITING SYSTEMS

There are four main families of engineered nucleases that have been used to insert, delete, or replace DNA in the genome: zinc finger nucleases (ZFNs),[26] meganucleases,[27,28] transcription activator-like effector nucleases (TALENs),[29] and CRISPR-associated nuclease Cas9 (CRISPR-Cas9).[30,31] While ZFNs, meganucleases, and TALENs recognize specific sequences in the genome through protein-DNA interactions, CRISPR-Cas9 nucleases are directed to a target site through simple base pairing to a complementary short guide RNA (gRNA) sequence. Once bound to a target site, these nucleases generate double-stranded breaks (DSBs) in the genome and enlist endogenous cellular pathways to repair the lesion. In the absence of a repair template, non-homologous end joining (NHEJ) pathways are activated to rapidly re-ligate the two ends of the DSB.[32] NHEJ is error prone, and small insertions or deletions (indels) at the DSB cut site are frequently incorporated.[33] These indels can create frameshift mutations or premature stop codons that result in gene knockout

https://doi.org/10.1016/j.ymthe.2021.09.026.

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Table 1. Benefits of a large animal model

	Small animals	Large animals
Examples	rodents, zebrafish, insects, nematodes	NHPs, pigs, dogs, sheep
Sampling	limited blood volumes; difficult to isolate and characterize secondary organs	larger blood volumes and organs for studies of cancer, infectious diseases, and muscular dystrophies
Relevant cellular subsets	incomplete humanization in xenogeneic models (e.g., challenges in generating human myeloid and red blood cells in humanized mice)[21]	often highly conserved versus human; availability of comparable mutations for modeling (e.g., SCID and DMD dogs)
Relevant anatomical structures	smaller and often not comparable to human (e.g., rodent retina lacks a macula)[22]	close resemblance to human immune system (NHP), circulatory system (pigs)[12–14]
Lifespan	short; limitation for long-term follow-up studies	longer lifespans enable tracking of edited cells for years/decades
Cost	low	high
Ease of manufacturing	labor-intensive process for humanized mice; limited availability of specific human stem cell sources; smaller size enables smaller-scale manufacturing of cell products	larger size facilitates test of manufacturing feasibility at scales closer to human
Route of administration	frequently not relevant to humans (e.g., intraperitoneal or tail vein in rodents)	intravenous and intramuscular dosing, central lines, and other routes highly comparable to humans
Infectious disease models	fewer human pathogens capable of infecting rodents and lower metazoans	broader susceptibility of large animals to human pathogens and closely related strains (e.g., HIV and SIV)[23,24]
Musculoskeletal disease models	due to shortened lifespan, shorter time frame to study muscle wasting phenotypes, which are often less severe than human phenotypes[25]	more clinically relevant progressive muscle wasting phenotypes, which can be studied over a longer lifespan

NHP, nonhuman primate; SCID, severe combined immunodeficiency.

if the DSB occurs within the coding region of a gene. If a repair template with homology to the flanking regions of the DSB is provided, homologous recombination (HR) pathways can facilitate targeted integration of the repair template at the DSB.[34] Researchers have relied on NHEJ and HDR pathways to engineer specific DNA manipulations to knock out aberrant genes, excise foreign DNA, correct disease-causing mutations, and integrate therapeutic genes.

GENE EDITING APPROACHES FOR HIV-1

Since the discovery of HIV 40 years ago, 77.5 million people have been infected with HIV and almost 35 million people have died following progression to acquired immunodeficiency syndrome (AIDS) (Joint United Nations Programme on HIV/AIDS [UNAIDS]; https://www.unaids.org/). While suppressive antiretroviral therapy (ART) can efficiently block HIV replication and substantially improve the life expectancy of HIV$^+$ individuals,[35] ART is not curative, and latent viral reservoirs persist.[36] These viral reservoirs can be reactivated if ART is interrupted, and thus ART is only effective at mitigating viral replication if strict adherence is maintained.[37–39] To date, there have only been two documented cases of functional cure, defined here as sustained virus remission in the absence of ART. Both individuals, termed the Berlin and London patients, received myeloablative conditioning and transfusion of allogeneic hematopoietic stem cells (HSCs) to treat hematological malignancies.[40–42] The transplanted HSCs harbored a homozygous loss-of-function deletion in the *CCR5* gene (*CCR5*Δ32), the primary coreceptor used by most strains of HIV to gain entry into CD4$^+$ cells.[43] Reconstitution of the hematopoietic system with CCR5 null HIV-resistant HSCs led to durable HIV clearance in the absence of ART.

These case studies have inspired a new functional HIV cure approach that focuses on genetic engineering of CCR5 in a patient's own (autologous) cells to confer resistance to HIV infection. Although highly complementary to large animal models of HIV persistence and cure, studies in humanized mouse models of HIV are more challenging, since humanization protocols are labor intensive and often result in incomplete development of all hematopoietic lineages (Table 1).[21] Furthermore, due to their small size, adequate amounts of blood and tissue samples from rodents are more difficult to obtain. NHPs are widely regarded as the gold standard animal model for HIV. Although HIV-1 does not efficiently infect macaque cells (reviewed in Thippeshappa et al.[44]), NHPs are susceptible to closely related simian immunodeficiency virus (SIV) and simian/human immunodeficiency virus (SHIV), displaying hallmark features of HIV infection including CD4$^+$ T cell depletion, progression to AIDS-like disease, and establishment of reactivatable latent reservoirs.[23,24] A key consideration for NHP models of HIV cure and other diseases, including COVID-19,[45] is the NHP species to apply, usually either rhesus macaques (*Macaca mulatta*), pigtail macaques (*Macaca nemestrina*), or cynomolgus macaques (*Macaca fascicularis*). Due to species-specific differences in the activity of lentiviral restriction factors, including TRIM5α, for example, pigtail macaque cells can be modified by the identical HIV-based lentiviral vectors that are used in patients, whereas rhesus macaque cells require specially engineered SIV-based lentiviral vectors.[46–50] Cynomolgus macaques are slightly smaller than rhesus or pigtail macaques; the Mauritian subspecies further possesses limited MHC diversity due to a geographical bottleneck event, which is advantageous for MHC-centric studies of the control of numerous pathogens, including HIV.[51] We have developed an autologous transplantation model to evaluate the function and engraftment potential of CCR5-edited HSCs that is applicable in multiple NHP species.[52] Using ZFNs we achieved up to 64% CCR5 editing in pigtail macaque hematopoietic stem and progenitor cells (HSPCs) and demonstrated low but durable engraftment and multilineage repopulation of CCR5-edited progeny following myeloablative conditioning and transplantation (Table 2). Translating this approach to a model of latent HIV infection using SHIV-infected pigtail macaques

Table 2. Overview of gene editing in large animal models

Disease	Animal/disease model	Strategy	*In vivo* or *ex vivo*	Target	Gene editing system	Delivery	Conditioning	Results	Reference
HIV	NHP (pigtail macaque)	inactivate *CCR5* in HSCs to enable HIV-resistant hematopoiesis	*ex vivo*	*CCR5* in HSPCs	ZFN	electroporation of ZFN mRNA	myeloablative (TBI)	64% *CCR5* editing in infusion product, 3%–5% long-term engraftment	[52]
HIV	SHIV+ NHP (pigtail macaque)	inactivate *CCR5* in HSCs to enable HIV-resistant hematopoiesis	*ex vivo*	*CCR5* in HSCs	ZFN	electroporation of ZFN mRNA	myeloablative (TBI)	∼50% *CCR5* editing in infusion product, 3%–4% long-term engraftment, trafficking to secondary lymphoid tissue, trends toward delayed viral rebound after ART removal	[53]
HIV	SIV+ NHP (rhesus macaque)	inactivate *CCR5* in HSCs to enable HIV-resistant hematopoiesis	*ex vivo*	*CCR5* in HSPCs	CRISPR (SpCas9)	SIV-based LV	non-myeloablative (busulfan)	<16% *CCR5* editing in infusion product, ∼1% long-term engraftment, all but one animal rebounded after ART removal	[54]
HIV	SHIV+ NHP (rhesus macaque)	inactivate *CCR5* in anti-HIV CAR T cells to confer HIV resistance and enable virus-specific effector function	*ex vivo*	*CCR5* in anti-HIV CAR T cells	CRISPR (SpCas9)	electroporation of CRISPR RNPs	none	<36% *CCR5* editing in infusion product	[55]
HIV	SIV+ NHP (rhesus macaque)	excise integrated proviral DNA in SIV-infected cells	*in vivo*	SIV proviral DNA in SIV-infected cells	CRISPR (SaCas9)	AAV9	none	up to 92% and 95% decrease in proviral DNA in blood and peripheral lymph nodes	[56]
SCD	NHP (rhesus macaque)	POC: correct point mutation in *HBB* that causes SCD via single base pair HDR conversion	*ex vivo*	*HBB* in HSCs	CRISPR	electroporation of CRISPR RNP + ssDNA donor template to recreate SCD point mutation via HDR	myeloablative (TBI)	17%–26% recapitulation of SCD mutation in infusion product, ∼1% long-term engraftment	[57]
SCD/β-thalassemia	NHP (pigtail macaque)	disrupt *BCL11A* in HSCs to reactivate fetal hemoglobin	*ex vivo*	*BCL11A* in HSCs	TALEN	electroporation of TALEN mRNA	myeloablative (TBI)	1.5% *BCL11A* editing in infusion product, 0.3%–0.4% long-term engraftment	[58]
SCD/β-thalassemia	NHP (rhesus macaque)	prevent *BCL11A* repression of fetal hemoglobin by disrupting BCL11A binding site in γ-globin promoter	*ex vivo*	*HBG* promoter in HSCs	CRISPR	electroporation of CRISPR RNPs	myeloablative (TBI)	75% editing and 39% recapitulation of HPFH mutation in infusion product, 8%–27% editing and 6%–18% HbF expression in PB cells >1 year after treatment	[59]
SCD/β-thalassemia	NHP (rhesus macaque)	disrupt the erythroid-specific *BCL11A* enhancer region to disable *BCL11A* in erythroid lineages and reactivate fetal hemoglobin	*ex vivo*	erythroid-specific *BCL11A* enhancer region in HSCs	CRISPR (SpCas9)	electroporation of CRISPR RNPs	myeloablative (TBI)	up to 85% editing in enhancer region in infusion product, but engraftment and γ-globin expression highly	[60]

(*Continued on next page*)

Table 2. *Continued*

Disease	Animal/disease model	Strategy	*In vivo* or *ex vivo*	Target	Gene editing system	Delivery	Conditioning	Results	Reference
								dependent on number of infused cells	
AML	NHP (rhesus macaque)	POC: inactivate *CD33* in HSPCs to establish CD33-deficient hematopoiesis and enable CD33-directed immunotherapy	*ex vivo*	*CD33* in HSPCs	CRISPR (SpCas9)	electroporation of CRISPR RNPs	myeloablative (TBI)	<15% *CD33* editing in infusion product, 2%–4% long-term engraftment	61
DMD	DeltaE50-MD dogs[62]	disrupt *DMD* exon 51 splice acceptor site to enable exon 51 skipping and restoration of dystrophin reading frame	*in vivo*	*DMD* exon 51 splice acceptor site in peripheral and cardiac muscle	CRISPR (SpCas9)	dual AAV9 to co-deliver Cas9 and gRNA	none	restoration of up to 70% and 92% of normal dystrophin in peripheral and cardiac muscles 8 weeks post-treatment	63
DMD	DMD exon 52-deficient pigs[64]	excise *DMD* exon 51 to restore dystrophin reading frame	*in vivo*	*DMD* exon 51 in peripheral and cardiac muscle	CRISPR (SpCas9)	dual AAV9 to deliver split intein Cas9 + gRNA	none	widespread expression of truncated dystrophin in cardiac and skeletal muscle, decreased fibrosis, improved cardiac function and survival	65
Hypercholesterolemia	NHP (rhesus macaque)	knock out *PCSK9* to prevent degradation of LDLR and increase uptake of blood LDL-c	*in vivo*	*PCSK9* in hepatocytes	meganuclease	AAV8	none	up to 84% reduction in serum PCSK9 and 60% LDL-c 11 months after treatment	66
Hypercholesterolemia	NHP (rhesus macaque)	knock out *PCSK9* to prevent degradation of LDLR and increase uptake of blood LDL-c	*in vivo*	*PCSK9* in hepatocytes	meganuclease	AAV8	none	sustained dose-dependent reductions in serum PCSK9 and LDL-c 3 years after treatment	67
Hypercholesterolemia	NHP (cynomolgus macaque)	introduce precise loss-of-function *PCSK9* mutation to knock out *PCSK9*, prevent LDLR degradation, and increase uptake of blood LDL-c	*in vivo*	*PCSK9* in hepatocytes	CRISPR adenine base editors	LNP delivery of ABE8.8 mRNA and PCSK9 gRNA	none	>60% *PCSK9* editing in NHP liver, stable 90% reduction of PCSK9 and 60% reduction of LDL-c	68
Hypercholesterolemia	NHP (cynomolgus macaque)	introduce precise loss-of-function *PCSK9* mutation to knock out *PCSK9*, prevent LDLR degradation, and increase uptake of blood LDL-c	*in vivo*	*PCSK9* in hepatocytes	CRISPR adenine base editors	LNP delivery of ABEmax mRNA and PCSK9 gRNA	none	up to 34% *PCSK9* editing in NHP liver, ∼32% reduction in PCSK9 and ∼14% reduction in LDL-c	69
Leber congenital amaurosis	NHP (cynomolgus macaque)	POC: correct aberrant splice donor created by mutation in *CEP290* to restore reading frame and normal *CEP290* expression	*in vivo*	*CEP290* mutation in retinal cells	CRISPR (SaCas9)	AAV5 delivery of SaCas9 and pair of gRNA	none	up to 30% reading frame-restoring editing	70
Cone-rod dystrophy (CORD6)	NHP (cynomolgus macaque)	POC: knockout of mutant *GUCY2D* followed by complementation with wt *GUCY2D*	*in vivo*	*GUCY2D* in retinal cells	CRISPR (SaCas9)	dual AAV5 delivery of SaCas9 and gRNA	none	10%–20% editing in photoreceptor cells, up to 80% decrease in *GUCY2D* protein product	71

suppressed with ART yielded comparable CCR5 editing efficiencies in HSCs and engraftment of CCR5-edited cells, notably in secondary lymphoid tissues known to harbor viral reservoirs.[53] While transplanted animals ultimately exhibited viral rebound after ART cessation, the time to viral rebound trended later in CCR5-edited HSC transplants relative to unedited HSC transplanted controls. This approach was also investigated in SIV-infected ART-suppressed rhesus macaques by Yu and colleagues.[54] *Ex vivo* CRISPR-Cas9 editing in HSPCs was suboptimal (<16%), and engraftment in busulfan-conditioned animals was low (~1%). While all but one animal exhibited viral rebound after ART withdrawal, a small increase in CCR5 editing was detected in all hematopoietic subsets analyzed. Together, these data suggest that a selective advantage is conferred in CCR5 null cells in the context of active viremia but highlight that more efficient means will be needed to achieve a therapeutic threshold of engraftment of CCR5-edited cells.[72]

Data from our group and others in the NHP model suggest that the successful cure of the Berlin and London patients was largely due to the near-complete hematopoietic reconstitution with donor-derived, HIV-resistant cells.[73–75] Since both patients were primarily treated for their hematological cancers, lymphodepletion via myeloablative conditioning was a justifiable risk, whereas for otherwise healthy ART-suppressed HIV$^+$ patients, the toxicities associated with myeloablative conditioning are generally not reasonable. While several clinical trials have attempted to recreate the success of the Berlin and London patients through transplantation of autologous CCR5-edited HSCs in non-conditioned patients, these studies have ultimately failed to eliminate HIV after ART withdrawal, since HIV-vulnerable CCR5$^+$ lymphocytes and latent viral reservoirs persist.[76–79] In order to enable ART-free remission of HIV, more active means of targeting these reservoirs will likely be necessary. Rather than replacing the entire immune system with CCR5-edited cells, for example, a smaller number of enhanced virus-specific immune effectors, like chimeric antigen receptor (CAR) T cells, could be applied. We recently provided proof of principle for this strategy by transplanting SHIV-infected rhesus macaques with T cells that were electroporated with CCR5-targeted CRISPR-Cas9 ribonucleoprotein (RNP) complexes and transduced with lentiviral vectors expressing a virus-specific CAR.[55] More elegant "2-for-1" gene editing strategies in which a virus-specific CAR is targeted to the CCR5 locus to enable simultaneous CAR integration and CCR5 knockout have also been developed. While these approaches showed promise in *ex vivo* studies and in small animal models, they remain to be tested in large animal models or in clinical studies.[80,81]

Gene editing to excise integrated proviral DNA has also shown promise in SIV-infected ART-suppressed rhesus macaque models. Through systemic delivery of adeno-associated virus serotype 9 (AAV9) vectors expressing *Staphylococcus aureus* Cas9 (SaCas9) and a pair of gRNAs targeting the SIV genome, Mancuso et al.[56] demonstrated efficient excision of integrated proviral DNA in blood and relevant secondary lymphoid tissue. Following a single infusion, intact SIV DNA was markedly reduced, with up to 95% decrease in SIV DNA in lymph nodes. Given that multiplexed genome editing and DSB generation can result in chromosomal translocations,[82,83] further studies evaluating the safety of this approach are required, particularly since AAV9 delivery of CRISPR machinery will lead to prolonged expression in broad tissue types and potential integration of the transgene at the cut site.[84] Additionally, whether this approach is capable of accessing every latently infected cell in the body and inactivating replication-competent provirus, which will be required in order to prevent viral rebound in the absence of ART, remains to be determined.

GENE EDITING FOR HEMOGLOBINOPATHIES

Hemoglobinopathies are the most common genetic diseases, with around 1.1% of all couples at risk for having a child with a hemoglobin disorder.[85] Sickle cell disease (SCD) and β-thalassemia are the most prevalent hemoglobinopathies and are caused by mutations in the β-globin gene (*HBB*), which together with α-globin forms adult hemoglobin (HbA). In SCD, a point mutation in *HBB* results in structural abnormalities in hemoglobin and the formation of sickle hemoglobin (HbS).[86] Patients homozygous for HbS develop fragile, sickle-shaped red blood cells that cause vaso-occlusion, pain crises, irreversible organ damage, and early mortality.[87] In β-thalassemia, mutations in *HBB* result in reduced or absent HBB synthesis, which leads to ineffective erythropoiesis and anemia.[88] Currently, allogeneic bone marrow transplantation to replace defective HSCs is the only cure for SCD and β-thalassemia, although this approach is hampered by scarcities in HLA-matched donors. While vector-based gene therapy approaches to supplement autologous HSCs with a corrected *HBB* gene prior to transplantation have shown success in clinical trials,[89,90] gene editing strategies offer the potential for endogenous gene correction without the risks associated with genomic integration of a viral vector.[91] NHP models are uniquely suited for evaluating gene-edited HSC therapeutics since, unlike mouse HSCs, NHP and human HSCs can be identified by characteristic CD34 expression,[92,93] enabling analogous and clinically relevant HSC isolation methods.[20] Furthermore, for recently developed HSC-targeted *in vivo* gene therapy technologies,[94,95] NHP models are useful for assessing genotoxicity, off-target transduction, and long-term efficacy.

For SCD, many groups have explored gene editing strategies that directly target and correct the causative mutation in the *HBB* coding sequence.[96–99] Similar to preclinical models of cancer, however, no large animal model of SCD currently exists. To demonstrate proof-of-principle correction of the SCD point mutation in *HBB*, Uchida et al.[57] used CRISPR-Cas9 to generate the SCD mutation via HDR in CD34$^+$ HSCs isolated from healthy rhesus macaques. HDR efficiency in infusion products was moderate (17%–26% gene conversion) and decreased to ~1% in peripheral lymphocytes and granulocytes 16 weeks after transplantation into conditioned NHPs. HbS production similarly declined over time, from 8%–17% at early time points to 3%–10% 12 months post-transplant.

An alternative approach that has shown promise in large animal studies focuses on restoring functional hemoglobin through the reactivation of fetal hemoglobin (HbF), a tetramer composed of two

α-globin and two γ-globin chains. While HbF is usually silenced after birth, post-natal HbF can be maintained or reactivated, as observed in individuals with a benign condition known as hereditary persistence of fetal hemoglobin (HPFH).[100] Importantly, increased levels of HbF are correlated with decreased morbidity and mortality in SCD and β-thalassemia patients.[101] HbF expression can be induced by inactivating BCL11A, a transcriptional repressor of HbF.[102] To test this strategy, we established an NHP transplantation model to evaluate HbF reactivation in autologous BCL11A-edited $CD34^+$ HSCs.[58] We subsequently used CRISPR-Cas9 to recapitulate a naturally occurring 13-nucleotide deletion in HPFH individuals that disrupts the BCL11A binding site found in the γ-globin promoter regions.[103,59] We achieved an average of 75% on-target editing and up to 39% recapitulation of the HPFH deletion either in $CD34^+$ cells or in the refined $CD90^+$ HSC enriched subpopulation. After myeloablative conditioning and transplantation, edited HSCs displayed stable, multilineage engraftment in rhesus macaques for over 1 year. Editing at the γ-globin locus was detected in 8% to 27% of peripheral blood (PB)-nucleated cells, and HbF-expressing cells stably reconstituted 6% to 18% of PB (Table 2).

Given that BCL11A plays critical roles in HSC self-renewal,[104] lymphoid development,[105] and dendritic cell fate,[106] inactivation of BCL11A in HSCs may impair normal hematopoiesis. In 2013, Bauer et al.[107] used genome-wide association studies to uncover an erythroid-specific BCL11A enhancer region that could be mutated in HSCs to inactivate BCL11A and induce HbF expression in erythroid cells.[108] This strategy was tested in an NHP model of autologous HSC editing and transplantation, using CRISPR-Cas9 to disrupt the erythroid-specific enhancer and restore HbF expression in rhesus macaques.[60] Infused HSCs demonstrated high (80%–85%) on-target editing, although engraftment and γ-globin expression were highly dependent on the number of cells infused. Targeting of the BCL11A enhancer region is also being explored in two clinical trials (NCT03655678, NCT03745287). Early reports from two patients—one with β-thalassemia and one with SCD—demonstrated ∼80% *ex vivo* editing in HSCs and >60% editing in nucleated PB cells more than 1 year after myeloablative conditioning and infusion.[109] HbF expression increased rapidly in both patients, with HbF accounting for >91% of total hemoglobin in the β-thalassemia patient and 43.2% in the SCD patient. Importantly, neither patient experienced any further vaso-occlusive episodes or required transfusion of packed red cells by 30 days post-infusion. While these data are preliminary and longer-term studies in more patients are required, these extremely promising early reports are likely to change the landscape of β-thalassemia and SCD treatment and serve as the flagship example of the potential of gene editing to cure human disease.

MODELING GENE EDITING THERAPIES FOR CANCER IN LARGE ANIMALS

CAR T cell therapies have shown remarkable responses in patients with B cell malignancies, as evidenced by recent US Food and Drug Administration (FDA) approvals.[110–114] However, the success of this approach is highly dependent on the availability of cancer-restricted surface markers that can be targeted by CAR T cells. Although validated models of tumorigenesis are not readily available, proof-of-principle experiments in healthy NHPs provide a useful model to quantify depletion in cellular subsets that normally express such markers. For example, CD20 CAR T cells designed to target B cell malignancies have been shown to efficiently induce $CD20^+$ B cell aplasia in healthy rhesus macaques.[115] In acute myeloid leukemia (AML), up to 90% of leukemic blasts express CD33, a sialic acid receptor, although shared CD33 expression on normal hematopoietic and myeloid progenitors poses challenges for AML-specific targeting.[116,117] While the role of CD33 in myeloid cells is not fully understood, CD33 knockout in mice showed no apparent consequence on survival, fertility, or hematopoiesis, suggesting CD33 may be dispensable for normal hematological function.[118] This finding opens the door to CD33 gene editing/immunotherapeutic strategies in HSCs, where reconstitution of CD33 null hematopoiesis may enable CD33-dependent selection in AML patients. While this concept has been successfully demonstrated in mouse AML xenograft models,[61,119] validation in an NHP model is critical, since long-term safety and engraftment cannot be assessed in a short-lived immunocompromised mouse model. In a proof-of-concept (POC) study, Kim et al.[61] demonstrated that CRISPR-Cas9 inactivation of CD33 in HSPCs had no impact on myeloid development or function after autologous transplantation into conditioned rhesus macaques. While on-target editing in the infusion product was low (<15%), the CD33-edited $CD34^+$ HSPCs engrafted and CD33 editing in myeloid cells and bone marrow HSPCs was stably detected for over 1 year. Follow-up studies assessing the efficacy of subsequent CD33-directed immunotherapy are required, although the lack of suitable NHP models of AML and other cancers may pose challenges.

GENE EDITING FOR DUCHENNE MUSCULAR DYSTROPHY

Duchenne muscular dystrophy (DMD) is a severe X-linked recessive disease that affects roughly 1 in 5,000 newborn males and is characterized by progressive muscle degeneration and atrophy.[120,121] DMD is caused by loss-of-function mutations in the gene that encodes dystrophin (*DMD*), a critical cytoskeletal protein.[122] Despite improvements in standard of care, most DMD patients die from respiratory and cardiac failure between 20 and 40 years of age.[123] While thousands of *DMD* mutations have been identified,[124] frameshifting mutational "hotspots" are commonly found across exons 45 to 53.[125] Dystrophin-deficient DMD animal models have been generated in mice,[126–132] rats,[133,134] and rabbits,[135] although DMD models in larger animals, including dogs,[62] pigs,[64,136] and rhesus macaques,[137] display phenotypes that more closely resemble human DMD pathophysiology.[25] As such, large animal DMD models are particularly useful for functional evaluation of novel therapeutics. Targeted exon skipping to restore the *DMD* reading frame and generate a truncated but functional dystrophin protein is an area of active research, particularly given that more than 90% of DMD-causing mutations are predicted to be suitable for exon-skipping strategies.[138] Moreover, a restoration of just 15% of the normal dystrophin levels is estimated to provide significant therapeutic improvement for DMD patients.[139,140]

Gene editing approaches to restore the *DMD* open reading frame and rescue functional dystrophin expression have recently been demonstrated in large animal models of DMD. Using CRISPR-Cas9 to target the *DMD* exon 51 splice acceptor site, Amoasii et al.[63] co-delivered AAV9-Cas9 and AAV9-gRNA to deltaE50-MD dogs. Dystrophin rescue varied by administration route, with systemic delivery achieving up to 70% and 92% of wild-type (wt) dystrophin levels 8 weeks post-treatment in the peripheral and cardiac muscles, respectively. More recently, Moretti et al.[65] demonstrated that dual AAV9s could be used to co-deliver a split-intein Cas9 and a pair of gRNAs to excise exon 51 and restore the reading frame in *DMD* exon 52-deficient pigs.[64,141] Intravenous delivery of AAV9 vectors coated in polyamidoamine nanoparticles resulted in efficient exon 51 targeting and robust expression of a shortened form of dystrophin in skeletal muscle, heart tissue, and the diaphragm. While the truncated dystrophin was only partially functional, treated pigs showed significant reductions in fibrosis, improvements in cardiac function, and prolonged survival. This work represents an important step toward functional validation of gene editing therapeutics for DMD, but further studies are needed to fine-tune vector dosing, cardiac targeting, and toxicity.

GENE EDITING FOR HYPERCHOLESTEROLEMIA

Genome editing has also shown promise for the treatment of elevated levels of cholesterol in the blood (hypercholesterolemia). Proprotein convertase subtilisin/kexin type 9 (PCSK9), a protein encoded by the *PCSK9* gene, binds to the hepatic low-density lipoprotein receptor (LDLR) and inhibits the uptake of LDL cholesterol (LDL-c) from the bloodstream by catalyzing the degradation of LDLR.[142,143] Gain-of-function mutations in *PCSK9* have been identified in individuals with familial hypercholesterolemia, an autosomal dominant condition that is characterized by increased serum LDL-c and an elevated risk of coronary heart disease (CHD).[144] Conversely, loss-of-function *PCSK9* mutations, which are found in 2%–3% of some populations, are associated with decreased levels of LDL-c and a significant (up to 88%) reduction in the risk of CHD.[145–147] While several antibody-based *PCSK9* inhibitors have gained FDA approval for the treatment of hypercholesterolemia,[148,149] gene editing approaches offer the potential for durable *PCSK9* inactivation from a single-dose treatment.

In 2018, Wang et al.[66] published the first report of *PCSK9* inhibition in rhesus macaques following a single infusion of AAV vectors expressing a *PCSK9*-targeted meganuclease. Serum PCSK9 and LDL-c decreased in a dose-dependent manner, with up to 84% PCSK9 and 60% LDL-c reduction 11 months after treatment. A 3-year follow-up report demonstrated durable PCSK9 and LDL-c reductions, with no significant changes in levels measured at year 2.[67] Off-target editing was low but detectable at up to 80 sites by inverted terminal repeat sequencing (ITR-seq),[150] and large (>15 bp) insertions at the *PCSK9* locus were identified, the majority of which contained portions of the vector transgene. While vector-associated immunotoxicity was only evident at early time points, the presence of integrated transgene at the *PCSK9* locus will likely raise safety concerns. A *PCSK9* inhibition approach that avoids generating DSBs may help address these safety issues. Recently, Musunuru et al.[68] demonstrated that CRISPR-derived adenine base editors (ABEs) could be used to introduce precise *PCSK9* loss-of-function mutations in cynomolgus macaques. Following treatment with a single intravenous infusion of lipid nanoparticles (LNPs) containing ABE8.8 mRNA and *PCSK9* gRNA,[151] NHPs showed a durable 90% reduction in blood PCSK9 and 60% reduction in serum LDL-c for up to 8 months. Of the 48 off-target sites interrogated by ONE-seq,[152] editing was only evident at one site. A similar approach using ABEmax[69] (in place of ABE8.8) and the same LNP formulation was also recently demonstrated in a cynomolgus macaque model, although on-target editing, PCSK9 knockout, and serum LDL-c reduction were less efficient.[153] While further studies are needed to assess the long-term safety and efficacy of ABE-mediated *PCSK9* inhibition, the reduction in off-target editing, as well as the absence of DSB generation, vector integration, and prolonged meganuclease expression may offer significant safety advantages.

GENE EDITING FOR INHERITED RETINAL DEGENERATIONS

Inherited retinal degenerations (IRDs) are a group of heterogeneous disorders characterized by degeneration of photoreceptors and retinal pigment epithelium.[154] IRDs are a leading cause of blindness, with a global incidence of ∼1 in 2,000.[155] IRD-causing mutations in more than 250 genes have been identified, and while some recessive IRD mutations can be treated with gene supplementation therapies,[156] many autosomal dominant IRD mutations are not amenable to these approaches, since gene supplementation cannot overcome the dominant effect of the mutated gene product. As such, gene editing therapies that correct the underlying IRD mutations are actively being explored. Notably, the eye is an ideal target for developing novel gene editing therapies: therapeutics can be easily administered at this site, and because the eye is an immunoprivileged organ, anti-transgene and anti-vector immune responses are less likely to occur. Major anatomical differences between the mouse and human eye have been characterized, including the lack of a macula in the mouse retina (Table 1). NHP retinal anatomy is highly comparable to humans, making NHPs the optimal animal model for evaluating novel IRD therapies.[22,157]

One IRD being targeted with gene editing is Leber congenital amaurosis (LCA), an autosomal recessive disorder that affects ∼1 in 40,000 newborns[158,159] and results in blindness or significant visual impairment in early infancy.[160] LCA is caused by deleterious mutations in genes involved in diverse aspects of retinal function, such as photoreceptor development and transduction, vitamin A cycling, and guanine synthesis.[161] While up to 18 genes have been implicated in LCA,[162] *CEP290*, *GUCY2D*, and *CRB1* are the most commonly mutated genes, occurring in 15%, 12%, and 10% of LCA patients, respectively.[161] In a proof-of-principle study, Maeder et al.[70] demonstrated that CRISPR-Cas9 could be used to correct the most prevalent loss-of-function mutation in *CEP290* that impairs photoreceptor function.[163] Healthy cynomolgus macaques were treated with AAV5 vectors expressing SaCas9 and a pair of gRNAs targeting the

flanking regions of the *CEP290* mutation site to excise the site or invert the intervening sequence within the *CEP290* reading frame. Importantly, only ~10% of functional photoreceptors are required for near-normal visual acuity.[164] Up to 30% productive editing (i.e., predicted to restore the reading frame) was achieved at the highest dose, exceeding the therapeutic threshold that is hypothesized to restore vision. This promising therapeutic is now being investigated in a clinical trial (NCT03872479), highlighting the direct translatability of findings from large animal models to the clinic.

GUCY2D is another gene that is frequently implicated in IRDs. While loss-of-function mutations in *GUCY2D* are common in LCA patients, gain-of-function mutations in *GUCY2D* are identified in 35% of patients with cone-rod dystrophy 6 (CORD6),[165,166] a rare autosomal dominant eye disorder. CORD6 pathology progresses more slowly than LCA, and patients typically present with macular degeneration, photophobia, and loss of visual and color acuity by age 10.[167] *GUCY2D* encodes retGC1, an enzyme that facilitates the recovery phase of phototransduction. In CORD6, *GUCY2D* mutations lead to increased retGC1 activity, aberrant calcium signaling, and photoreceptor apoptosis. Inactivation of mutant *GUCY2D* followed by rescue with wt *GUCY2D* is one treatment strategy for CORD6 that is actively being investigated. In a proof-of-concept study, McCullough et al.[71] co-injected dual AAV5 vectors expressing SaCas9 and *GUCY2D* gRNA into the retina of healthy cynomolgus macaques and detected up to 20% editing in photoreceptor cells and ~80% reduction in retGC1 expression. This study represents an important step toward correcting *GUCY2D*-mediated CORD6, though future studies evaluating the success of subsequent rescue with wt *GUCY2D* are needed.

CONCLUSIONS AND FUTURE DIRECTIONS

Compared to studies in small animals, therapeutic validation in large animals is more likely to be predictive of clinical success.[168] As such, use of large animal disease models will be critical as the gene editing field continues to flourish. Moving forward, large animal models will continue to provide a key resource, for example, to optimize cell manufacturing at a scale that is most relevant to a patient. This involves not only an increase of several orders of magnitude in cell number and associated gene editing reagents (e.g., CRISPR RNP), but also the maintenance of an optimal ratio of gene editing efficiency to toxicity. Associated instrumentation (e.g., electroporation devices) must be capable of adjusting over this scale and should ideally transition seamlessly from the handling of millions to hundreds of millions or billions of cells. Previously, we observed a sharp decrease in editing efficiency when increasing to NHP scale for delivery of BCL11A-specific TALEN mRNA electroporation, despite no impact on delivery of a control GFP mRNA.[58] We have recently found that in addition to limitations associated with electroporation hardware platforms, mRNA stability may also become an increasingly relevant factor as larger numbers of cells are handled in a given experiment (C.W.P. and R. Venkataraman, unpublished data). Stabilized mRNA formulations, transitioning to pre-formed, enzymatically active complexes like CRISPR RNPs, and use of electroporation devices featuring microfluidic and flowthrough capabilities[169] are the most likely paths forward to overcome barriers related to scale-up for gene editing experiments in large animal models and in early-phase clinical studies. More importantly, so-called *in vivo* delivery approaches that prioritize global health endpoints by increasing portability and scalability should be a key focus moving forward. These strategies are needed in resource-limited settings where expensive gene therapy infrastructure is not available. For some of the indications described in this review, *in vivo* delivery systems are already at the forefront—for example, AAV vectors to treat DMD, hypercholesterolemia, and IRDs. For the treatment of other diseases, including HIV-1,[170] rapidly emerging pathogens such as SARS-CoV-2,[171] SCD,[172] and cancer,[173] bringing effective treatments to the patient without the need for costly cell manufacturing facilities will be more challenging. While AAV vectors are a major platform for *in vivo* gene delivery, adenoviral vectors and nanoparticle-based approaches have emerged as particularly promising alternatives. Gutless adenoviral vectors feature large genetic packaging capacities, which can be used to deliver both CRISPR-Cas9 machinery and homology donor templates to a cell type of interest.[174] Nanoparticle formulations, namely mRNA-loaded LNPs, are leading global SARS-CoV-2 vaccination efforts and are exquisitely programmable and targetable for other indications, including cancer and metabolic diseases.[175,176] Looking forward, these *in vivo* delivery platforms hold great promise in targeting a broadly neutralizing antibody to the inactivated CCR5 locus for HIV cure, or precise repair of sickle cell mutations without the need for *ex vivo* HSPC therapy, all via a single intravenous injection in an outpatient setting. While large animal studies are more costly and require specialized veterinary staff, the benefits of testing therapeutics and novel *in vivo* delivery systems in animal models that bear greater resemblance to human physiology, anatomy, and immunology should not be understated. Identifying critical issues in long-term safety, feasibility, and efficacy before novel genome editing therapies enter the clinic will help to de-risk these approaches, optimize formulations and dosing, and potentially increase the chance of clinical success.

ACKNOWLEDGMENTS

We thank Helen Crawford for help in preparing this review and acknowledge the work of numerous colleagues whose published studies could not be cited due to space constraints. The authors are supported by grants from the NIH National Institute of Allergy and Infectious Diseases (UM1 AI126623, R01 AI135953, U01 AI138329, U19 AI149680, and U19 AI149505) and National Heart, Lung, and Blood Institute (R01 HL136135, U19 HL156247, and R01 HL151765).

DECLARATION OF INTERESTS

H.P.K. has received support as the inaugural recipient of the José Carreras/E. Donnall Thomas Endowed Chair for Cancer Research and the Stephanus Family Endowed Chair for Cell and Gene Therapy, and is or was a consultant to and has or had ownership interests with Rocket Pharmaceuticals, Homology Medicines, VOR Biopharma and Ensoma Inc. H.P.K. has also been a consultant to CSL Behring and Magenta Therapeutics. Other authors have no competing interests.

REFERENCES

1. Seok, J., Warren, H.S., Cuenca, A.G., Mindrinos, M.N., Baker, H.V., Xu, W., Richards, D.R., McDonald-Smith, G.P., Gao, H., Hennessy, L., et al.; Inflammation and Host Response to Injury, Large Scale Collaborative Research Program (2013). Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc. Natl. Acad. Sci. USA *110*, 3507–3512.
2. Parekh, C., and Crooks, G.M. (2013). Critical differences in hematopoiesis and lymphoid development between humans and mice. J. Clin. Immunol. *33*, 711–715.
3. Voskoglou-Nomikos, T., Pater, J.L., and Seymour, L. (2003). Clinical predictive value of the in vitro cell line, human xenograft, and mouse allograft preclinical cancer models. Clin. Cancer Res. *9*, 4227–4239.
4. Begley, C.G., and Ellis, L.M. (2012). Drug development: Raise standards for preclinical cancer research. Nature *483*, 531–533.
5. Cavanaugh, S.E., Pippin, J.J., and Barnard, N.D. (2014). Animal models of Alzheimer disease: historical pitfalls and a path forward. ALTEX *31*, 279–302.
6. Benatar, M. (2007). Lost in translation: treatment trials in the SOD1 mouse and in human ALS. Neurobiol. Dis. *26*, 1–13.
7. Roep, B.O., Atkinson, M., and von Herrath, M. (2004). Satisfaction (not) guaranteed: re-evaluating the use of animal models of type 1 diabetes. Nat. Rev. Immunol. *4*, 989–997.
8. van der Worp, H.B., Howells, D.W., Sena, E.S., Porritt, M.J., Rewell, S., O'Collins, V., and Macleod, M.R. (2010). Can animal models of disease reliably inform human studies? PLoS Med. *7*, e1000245.
9. Dehoux, J.P., and Gianello, P. (2007). The importance of large animal models in transplantation. Front. Biosci. *12*, 4864–4880.
10. Meurens, F., Summerfield, A., Nauwynck, H., Saif, L., and Gerdts, V. (2012). The pig: a model for human infectious diseases. Trends Microbiol. *20*, 50–57.
11. Swindle, M.M., Makin, A., Herron, A.J., Clubb, F.J., Jr., and Frazier, K.S. (2012). Swine as models in biomedical research and toxicology testing. Vet. Pathol. *49*, 344–356.
12. Phillips, K.A., Bales, K.L., Capitanio, J.P., Conley, A., Czoty, P.W., 't Hart, B.A., Hopkins, W.D., Hu, S.L., Miller, L.A., Nader, M.A., et al. (2014). Why primate models matter. Am. J. Primatol. *76*, 801–827.
13. Tsang, H.G., Rashdan, N.A., Whitelaw, C.B., Corcoran, B.M., Summers, K.M., and MacRae, V.E. (2016). Large animal models of cardiovascular disease. Cell Biochem. Funct. *34*, 113–132.
14. Skaggs, H., Chellman, G.J., Collinge, M., Enright, B., Fuller, C.L., Krayer, J., Sivaraman, L., and Weinbauer, G.F. (2019). Comparison of immune system development in nonclinical species and humans: Closing information gaps for immunotoxicity testing and human translatability. Reprod. Toxicol. *89*, 178–188.
15. AlJanahi, A.A., Lazzarotto, C.R., Chen, S., Shin, T.H., Cordes, S., Fan, X., Jabara, I., Zhou, Y., Young, D.J., Lee, B.C., et al. (2021). Prediction and validation of hematopoietic stem and progenitor cell off-target editing in transplanted rhesus macaques. Mol. Ther. , Published online June 24, 2021. https://doi.org/10.1016/j.ymthe.2021.06.016.
16. Ladiges, W.C., Storb, R., and Thomas, E.D. (1990). Canine models of bone marrow transplantation. Lab. Anim. Sci. *40*, 11–15.
17. Sargan, D.R. (2004). IDID: inherited diseases in dogs: web-based information for canine inherited disease genetics. Mamm. Genome *15*, 503–506.
18. Rowell, J.L., McCarthy, D.O., and Alvarez, C.E. (2011). Dog models of naturally occurring cancer. Trends Mol. Med. *17*, 380–388.
19. Camacho, P., Fan, H., Liu, Z., and He, J.Q. (2016). Large mammalian animal models of heart disease. J. Cardiovasc. Dev. Dis. *3*, E30.
20. Trobridge, G.D., and Kiem, H.P. (2010). Large animal models of hematopoietic stem cell gene therapy. Gene Ther. *17*, 939–948.
21. Marsden, M.D. (2020). Benefits and limitations of humanized mice in HIV persistence studies. Retrovirology *17*, 7.
22. Marmorstein, A.D., and Marmorstein, L.Y. (2007). The challenge of modeling macular degeneration in mice. Trends Genet. *23*, 225–231.
23. Hatziioannou, T., and Evans, D.T. (2012). Animal models for HIV/AIDS research. Nat. Rev. Microbiol. *10*, 852–867.
24. Evans, D.T., and Silvestri, G. (2013). Nonhuman primate models in AIDS research. Curr. Opin. HIV AIDS *8*, 255–261.
25. McGreevy, J.W., Hakim, C.H., McIntosh, M.A., and Duan, D. (2015). Animal models of Duchenne muscular dystrophy: from basic mechanisms to gene therapy. Dis. Model. Mech. *8*, 195–213.
26. Kim, Y.G., Cha, J., and Chandrasegaran, S. (1996). Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain. Proc. Natl. Acad. Sci. USA *93*, 1156–1160.
27. Silva, G., Poirot, L., Galetto, R., Smith, J., Montoya, G., Duchateau, P., and Pâques, F. (2011). Meganucleases and other tools for targeted genome engineering: perspectives and challenges for gene therapy. Curr. Gene Ther. *11*, 11–27.
28. Arnould, S., Delenda, C., Grizot, S., Desseaux, C., Pâques, F., Silva, G.H., and Smith, J. (2011). The I-CreI meganuclease and its engineered derivatives: applications from cell modification to gene therapy. Protein Eng. Des. Sel. *24*, 27–31.
29. Joung, J.K., and Sander, J.D. (2013). TALENs: a widely applicable technology for targeted genome editing. Nat. Rev. Mol. Cell Biol. *14*, 49–55.
30. Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A., and Charpentier, E. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science *337*, 816–821.
31. Ran, F.A., Hsu, P.D., Wright, J., Agarwala, V., Scott, D.A., and Zhang, F. (2013). Genome engineering using the CRISPR-Cas9 system. Nat. Protoc. *8*, 2281–2308.
32. Jackson, S.P., and Bartek, J. (2009). The DNA-damage response in human biology and disease. Nature *461*, 1071–1078.
33. Lieber, M.R. (2010). The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. Annu. Rev. Biochem. *79*, 181–211, 10.
34. Bibikova, M., Beumer, K., Trautman, J.K., and Carroll, D. (2003). Enhancing gene targeting with designed zinc finger nucleases. Science *300*, 764.
35. Teeraananchai, S., Kerr, S.J., Amin, J., Ruxrungtham, K., and Law, M.G. (2017). Life expectancy of HIV-positive people after starting combination antiretroviral therapy: a meta-analysis. HIV Med. *18*, 256–266.
36. Finzi, D., Hermankova, M., Pierson, T., Carruth, L.M., Buck, C., Chaisson, R.E., Quinn, T.C., Chadwick, K., Margolick, J., Brookmeyer, R., et al. (1997). Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science *278*, 1295–1300.
37. Chun, T.W., Davey, R.T., Jr., Engel, D., Lane, H.C., and Fauci, A.S. (1999). Re-emergence of HIV after stopping therapy. Nature *401*, 874–875.
38. Davey, R.T., Jr., Bhat, N., Yoder, C., Chun, T.W., Metcalf, J.A., Dewar, R., Natarajan, V., Lempicki, R.A., Adelsberger, J.W., Miller, K.D., et al. (1999). HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression. Proc. Natl. Acad. Sci. USA *96*, 15109–15114.
39. Deeks, S.G., Lewin, S.R., Ross, A.L., Ananworanich, J., Benkirane, M., Cannon, P., Chomont, N., Douek, D., Lifson, J.D., Lo, Y.R., et al.; International AIDS Society Towards a Cure Working Group (2016). International AIDS Society global scientific strategy: towards an HIV cure 2016. Nat. Med. *22*, 839–850.
40. Hütter, G., Nowak, D., Mossner, M., Ganepola, S., Müssig, A., Allers, K., Schneider, T., Hofmann, J., Kücherer, C., Blau, O., et al. (2009). Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. N. Engl. J. Med. *360*, 692–698.
41. Allers, K., Hütter, G., Hofmann, J., Loddenkemper, C., Rieger, K., Thiel, E., and Schneider, T. (2011). Evidence for the cure of HIV infection by CCR5Δ32/Δ32 stem cell transplantation. Blood *117*, 2791–2799.
42. Gupta, R.K., Peppa, D., Hill, A.L., Gálvez, C., Salgado, M., Pace, M., McCoy, L.E., Griffith, S.A., Thornhill, J., Alrubayyi, A., et al. (2020). Evidence for HIV-1 cure after CCR5Δ32/Δ32 allogeneic haemopoietic stem-cell transplantation 30 months post analytical treatment interruption: a case report. Lancet HIV *7*, e340–e347.
43. Farzan, M., Mirzabekov, T., Kolchinsky, P., Wyatt, R., Cayabyab, M., Gerard, N.P., Gerard, C., Sodroski, J., and Choe, H. (1999). Tyrosine sulfation of the amino terminus of CCR5 facilitates HIV-1 entry. Cell *96*, 667–676.
44. Thippeshappa, R., Kimata, J.T., and Kaushal, D. (2020). Toward a macaque model of HIV-1 infection: Roadblocks, progress, and future strategies. Front. Microbiol. *11*, 882.

45. Salguero, F.J., White, A.D., Slack, G.S., Fotheringham, S.A., Bewley, K.R., Gooch, K.E., Longet, S., Humphries, H.E., Watson, R.J., Hunter, L., et al. (2021). Comparison of rhesus and cynomolgus macaques as an infection model for COVID-19. Nat. Commun. *12*, 1260.

46. Dietrich, E.A., Jones-Engel, L., and Hu, S.L. (2010). Evolution of the antiretroviral restriction factor TRIMCyp in Old World primates. PLoS ONE *5*, e14019.

47. Brennan, G., Kozyrev, Y., Kodama, T., and Hu, S.L. (2007). Novel TRIM5 isoforms expressed by Macaca nemestrina. J. Virol. *81*, 12210–12217.

48. Uchida, N., Hsieh, M.M., Washington, K.N., and Tisdale, J.F. (2013). Efficient transduction of human hematopoietic repopulating cells with a chimeric HIV1-based vector including SIV capsid. Exp. Hematol. *41*, 779–788.e1.

49. Evans, M.E., Kumkhaek, C., Hsieh, M.M., Donahue, R.E., Tisdale, J.F., and Uchida, N. (2014). TRIM5α variations influence transduction efficiency with lentiviral vectors in both human and rhesus CD34(+) cells in vitro and in vivo. Mol. Ther. *22*, 348–358.

50. Hanawa, H., Hematti, P., Keyvanfar, K., Metzger, M.E., Krouse, A., Donahue, R.E., Kepes, S., Gray, J., Dunbar, C.E., Persons, D.A., and Nienhuis, A.W. (2004). Efficient gene transfer into rhesus repopulating hematopoietic stem cells using a simian immunodeficiency virus-based lentiviral vector system. Blood *103*, 4062–4069.

51. Wiseman, R.W., Karl, J.A., Bohn, P.S., Nimityongskul, F.A., Starrett, G.J., and O'Connor, D.H. (2013). Haplessly hoping: macaque major histocompatibility complex made easy. ILAR J. *54*, 196–210.

52. Peterson, C.W., Wang, J., Norman, K.K., Norgaard, Z.K., Humbert, O., Tse, C.K., Yan, J.J., Trimble, R.G., Shivak, D.A., Rebar, E.J., et al. (2016). Long-term multilineage engraftment of autologous genome-edited hematopoietic stem cells in nonhuman primates. Blood *127*, 2416–2426.

53. Peterson, C.W., Wang, J., Deleage, C., Reddy, S., Kaur, J., Polacino, P., Reik, A., Huang, M.L., Jerome, K.R., Hu, S.L., et al. (2018). Differential impact of transplantation on peripheral and tissue-associated viral reservoirs: Implications for HIV gene therapy. PLoS Pathog. *14*, e1006956.

54. Yu, S., Ou, Y., Xiao, H., Li, J., Adah, D., Liu, S., Zhao, S., Qin, L., Yao, Y., and Chen, X. (2020). Experimental treatment of SIV-infected macaques via autograft of CCR5-disrupted hematopoietic stem and progenitor cells. Mol. Ther. Methods Clin. Dev. *17*, 520–531.

55. Rust, B.J., Kean, L.S., Colonna, L., Brandenstein, K.E., Poole, N.H., Obenza, W., Enstrom, M.R., Maldini, C.R., Ellis, G.I., Fennessey, C.M., et al. (2020). Robust expansion of HIV CAR T cells following antigen boosting in ART-suppressed nonhuman primates. Blood *136*, 1722–1734.

56. Mancuso, P., Chen, C., Kaminski, R., Gordon, J., Liao, S., Robinson, J.A., Smith, M.D., Liu, H., Sariyer, I.K., Sariyer, R., et al. (2020). CRISPR based editing of SIV proviral DNA in ART treated non-human primates. Nat. Commun. *11*, 6065.

57. Uchida, N., Li, L., Nassehi, T., Drysdale, C.M., Yapundich, M., Gamer, J., Haro-Mora, J.J., Demirci, S., Leonard, A., Bonifacino, A.C., et al. (2021). Preclinical evaluation for engraftment of $CD34^+$ cells gene-edited at the sickle cell disease locus in xenograft mouse and non-human primate models. Cell Rep. Med. *2*, 100247.

58. Humbert, O., Peterson, C.W., Norgaard, Z.K., Radtke, S., and Kiem, H.P. (2017). A Nonhuman Primate Transplantation Model to Evaluate Hematopoietic Stem Cell Gene Editing Strategies for β-Hemoglobinopathies. Mol. Ther. Methods Clin. Dev. *8*, 75–86.

59. Humbert, O., Radtke, S., Samuelson, C., Carrillo, R.R., Perez, A.M., Reddy, S.S., Lux, C., Pattabhi, S., Schefter, L.E., Negre, O., et al. (2019). Therapeutically relevant engraftment of a CRISPR-Cas9-edited HSC-enriched population with HbF reactivation in nonhuman primates. Sci. Transl. Med. *11*, eaaw3768.

60. Demirci, S., Zeng, J., Wu, Y., Uchida, N., Shen, A.H., Pellin, D., Gamer, J., Yapundich, M., Drysdale, C., Bonanno, J., et al. (2020). BCL11A enhancer-edited hematopoietic stem cells persist in rhesus monkeys without toxicity. J. Clin. Invest. *130*, 6677–6687.

61. Kim, M.Y., Yu, K.R., Kenderian, S.S., Ruella, M., Chen, S., Shin, T.H., Aljanahi, A.A., Schreeder, D., Klichinsky, M., Shestova, O., et al. (2018). Genetic inactivation of CD33 in hematopoietic stem cells to enable CAR T cell immunotherapy for acute myeloid leukemia. Cell *173*, 1439–1453.e19.

62. Walmsley, G.L., Arechavala-Gomeza, V., Fernandez-Fuente, M., Burke, M.M., Nagel, N., Holder, A., Stanley, R., Chandler, K., Marks, S.L., Muntoni, F., et al. (2010). A duchenne muscular dystrophy gene hot spot mutation in dystrophin-deficient cavalier king charles spaniels is amenable to exon 51 skipping. PLoS ONE *5*, e8647.

63. Amoasii, L., Hildyard, J.C.W., Li, H., Sanchez-Ortiz, E., Mireault, A., Caballero, D., Harron, R., Stathopoulou, T.R., Massey, C., Shelton, J.M., et al. (2018). Gene editing restores dystrophin expression in a canine model of Duchenne muscular dystrophy. Science *362*, 86–91.

64. Klymiuk, N., Blutke, A., Graf, A., Krause, S., Burkhardt, K., Wuensch, A., Krebs, S., Kessler, B., Zakhartchenko, V., Kurome, M., et al. (2013). Dystrophin-deficient pigs provide new insights into the hierarchy of physiological derangements of dystrophic muscle. Hum. Mol. Genet. *22*, 4368–4382.

65. Moretti, A., Fonteyne, L., Giesert, F., Hoppmann, P., Meier, A.B., Bozoglu, T., Baehr, A., Schneider, C.M., Sinnecker, D., Klett, K., et al. (2020). Somatic gene editing ameliorates skeletal and cardiac muscle failure in pig and human models of Duchenne muscular dystrophy. Nat. Med. *26*, 207–214.

66. Wang, L., Smith, J., Breton, C., Clark, P., Zhang, J., Ying, L., Che, Y., Lape, J., Bell, P., Calcedo, R., et al. (2018). Meganuclease targeting of PCSK9 in macaque liver leads to stable reduction in serum cholesterol. Nat. Biotechnol. *36*, 717–725.

67. Wang, L., Breton, C., Warzecha, C.C., Bell, P., Yan, H., He, Z., White, J., Zhu, Y., Li, M., Buza, E.L., et al. (2021). Long-term stable reduction of low-density lipoprotein in nonhuman primates following in vivo genome editing of PCSK9. Mol. Ther. *29*, 2019–2029.

68. Musunuru, K., Chadwick, A.C., Mizoguchi, T., Garcia, S.P., DeNizio, J.E., Reiss, C.W., Wang, K., Iyer, S., Dutta, C., Clendaniel, V., et al. (2021). In vivo CRISPR base editing of PCSK9 durably lowers cholesterol in primates. Nature *593*, 429–434.

69. Rothgangl, T., Dennis, M.K., Lin, P.J.C., Oka, R., Witzigmann, D., Villiger, L., Qi, W., Hruzova, M., Kissling, L., Lenggenhager, D., et al. (2021). In vivo adenine base editing of PCSK9 in macaques reduces LDL cholesterol levels. Nat. Biotechnol. *39*, 949–957.

70. Maeder, M.L., Stefanidakis, M., Wilson, C.J., Baral, R., Barrera, L.A., Bounoutas, G.S., Bumcrot, D., Chao, H., Ciulla, D.M., DaSilva, J.A., et al. (2019). Development of a gene-editing approach to restore vision loss in Leber congenital amaurosis type 10. Nat. Med. *25*, 229–233.

71. McCullough, K.T., Boye, S.L., Fajardo, D., Calabro, K., Peterson, J.J., Strang, C.E., Chakraborty, D., Gloskowski, S., Haskett, S., Samuelsson, S., et al. (2019). Somatic gene editing of GUCY2D by AAV-CRISPR/Cas9 alters retinal structure and function in mouse and macaque. Hum. Gene Ther. *30*, 571–589.

72. Cardozo-Ojeda, E.F., Duke, E.R., Peterson, C.W., Reeves, D.B., Mayer, B.T., Kiem, H.P., and Schiffer, J.T. (2021). Thresholds for post-rebound SHIV control after CCR5 gene-edited autologous hematopoietic cell transplantation. eLife *10*, e57646.

73. Mavigner, M., Watkins, B., Lawson, B., Lee, S.T., Chahroudi, A., Kean, L., and Silvestri, G. (2014). Persistence of virus reservoirs in ART-treated SHIV-infected rhesus macaques after autologous hematopoietic stem cell transplant. PLoS Pathog. *10*, e1004406.

74. Peterson, C.W., Benne, C., Polacino, P., Kaur, J., McAllister, C.E., Filali-Mouhim, A., Obenza, W., Pecor, T.A., Huang, M.L., Baldessari, A., et al. (2017). Loss of immune homeostasis dictates SHIV rebound after stem-cell transplantation. JCI Insight *2*, e91230.

75. Colonna, L., Peterson, C.W., Schell, J.B., Carlson, J.M., Tkachev, V., Brown, M., Yu, A., Reddy, S., Obenza, W.M., Nelson, V., et al. (2018). Evidence for persistence of the SHIV reservoir early after MHC haploidentical hematopoietic stem cell transplantation. Nat. Commun. *9*, 4438.

76. Tebas, P., Stein, D., Tang, W.W., Frank, I., Wang, S.Q., Lee, G., Spratt, S.K., Surosky, R.T., Giedlin, M.A., Nichol, G., et al. (2014). Gene editing of CCR5 in autologous CD4 T cells of persons infected with HIV. N. Engl. J. Med. *370*, 901–910.

77. Xu, L., Wang, J., Liu, Y., Xie, L., Su, B., Mou, D., Wang, L., Liu, T., Wang, X., Zhang, B., et al. (2019). CRISPR-edited stem cells in a patient with HIV and acute lymphocytic leukemia. N. Engl. J. Med. *381*, 1240–1247.

78. Tebas, P., Jadlowsky, J.K., Shaw, P.A., Tian, L., Esparza, E., Brennan, A.L., Kim, S., Naing, S.Y., Richardson, M.W., Vogel, A.N., et al. (2021). CCR5-edited CD4+ T cells augment HIV-specific immunity to enable post-rebound control of HIV replication. J. Clin. Invest. *131*, 144486.

79. Zeidan, J., Sharma, A.A., Lee, G., Raad, A., Fromentin, R., Fourati, S., Ghneim, K., Sanchez, G.P., Benne, C., Canderan, G., et al. (2021). Infusion of CCR5 gene-edited T cells allows immune reconstitution, HIV reservoir decay, and long-term virological control. bioRxiv. https://doi.org/10.1101/2021.1102.1128.433290.

80. Sather, B.D., Romano Ibarra, G.S., Sommer, K., Curinga, G., Hale, M., Khan, I.F., Singh, S., Song, Y., Gwiazda, K., Sahni, J., et al. (2015). Efficient modification of CCR5 in primary human hematopoietic cells using a megaTAL nuclease and AAV donor template. Sci. Transl. Med. *7*, 307ra156.

81. Hale, M., Mesojednik, T., Romano Ibarra, G.S., Sahni, J., Bernard, A., Sommer, K., Scharenberg, A.M., Rawlings, D.J., and Wagner, T.A. (2017). Engineering HIV-resistant, anti-HIV chimeric antigen receptor T cells. Mol. Ther. *25*, 570–579.

82. Shin, H.Y., Wang, C., Lee, H.K., Yoo, K.H., Zeng, X., Kuhns, T., Yang, C.M., Mohr, T., Liu, C., and Hennighausen, L. (2017). CRISPR/Cas9 targeting events cause complex deletions and insertions at 17 sites in the mouse genome. Nat. Commun. *8*, 15464.

83. Webber, B.R., Lonetree, C.L., Kluesner, M.G., Johnson, M.J., Pomeroy, E.J., Diers, M.D., Lahr, W.S., Draper, G.M., Slipek, N.J., Smeester, B.A., et al. (2019). Highly efficient multiplex human T cell engineering without double-strand breaks using Cas9 base editors. Nat. Commun. *10*, 5222.

84. Hanlon, K.S., Kleinstiver, B.P., Garcia, S.P., Zaborowski, M.P., Volak, A., Spirig, S.E., Muller, A., Sousa, A.A., Tsai, S.Q., Bengtsson, N.E., et al. (2019). High levels of AAV vector integration into CRISPR-induced DNA breaks. Nat. Commun. *10*, 4439.

85. Modell, B., and Darlison, M. (2008). Global epidemiology of haemoglobin disorders and derived service indicators. Bull. World Health Organ. *86*, 480–487.

86. Rees, D.C., Williams, T.N., and Gladwin, M.T. (2010). Sickle-cell disease. Lancet *376*, 2018–2031.

87. Steinberg, M.H. (1999). Management of sickle cell disease. N. Engl. J. Med. *340*, 1021–1030.

88. Cao, A., and Galanello, R. (2010). Beta-thalassemia. Genet. Med. *12*, 61–76.

89. Ribeil, J.A., Hacein-Bey-Abina, S., Payen, E., Magnani, A., Semeraro, M., Magrin, E., Caccavelli, L., Neven, B., Bourget, P., El Nemer, W., et al. (2017). Gene therapy in a patient with sickle cell disease. N. Engl. J. Med. *376*, 848–855.

90. Thompson, A.A., Walters, M.C., Kwiatkowski, J., Rasko, J.E.J., Ribeil, J.A., Hongeng, S., Magrin, E., Schiller, G.J., Payen, E., Semeraro, M., et al. (2018). Gene Therapy in Patients with Transfusion-Dependent β-Thalassemia. N. Engl. J. Med. *378*, 1479–1493.

91. Cavazzana-Calvo, M., Payen, E., Negre, O., Wang, G., Hehir, K., Fusil, F., Down, J., Denaro, M., Brady, T., Westerman, K., et al. (2010). Transfusion independence and HMGA2 activation after gene therapy of human β-thalassaemia. Nature *467*, 318–322.

92. Okuno, Y., Iwasaki, H., Huettner, C.S., Radomska, H.S., Gonzalez, D.A., Tenen, D.G., and Akashi, K. (2002). Differential regulation of the human and murine CD34 genes in hematopoietic stem cells. Proc. Natl. Acad. Sci. USA *99*, 6246–6251.

93. Brehm, M.A., Shultz, L.D., Luban, J., and Greiner, D.L. (2013). Overcoming current limitations in humanized mouse research. J. Infect. Dis. *208* (*Suppl 2*), S125–S130.

94. Li, C., Wang, H., Gil, S., Georgakopoulou, A., Radtke, S., Yannaki, E., Papayannopoulou, T., Kiem, H.P., and Lieber, A. (2020). In vivo HSC gene therapy for hemoglobinopathies: A proof of concept evaluation in rhesus macaques. Blood *136*, 46–47.

95. Cannon, P., Asokan, A., Czechowicz, A., Hammond, P., Kohn, D.B., Lieber, A., Malik, P., Marks, P., Porteus, M., Verhoeyen, E., et al. (2021). Safe and effective in vivo targeting and gene editing in hematopoietic stem cells: strategies for accelerating development. Hum. Gene Ther. *32*, 31–42.

96. Hoban, M.D., Cost, G.J., Mendel, M.C., Romero, Z., Kaufman, M.L., Joglekar, A.V., Ho, M., Lumaquin, D., Gray, D., Lill, G.R., et al. (2015). Correction of the sickle cell disease mutation in human hematopoietic stem/progenitor cells. Blood *125*, 2597–2604.

97. Hoban, M.D., Lumaquin, D., Kuo, C.Y., Romero, Z., Long, J., Ho, M., Young, C.S., Mojadidi, M., Fitz-Gibbon, S., Cooper, A.R., et al. (2016). CRISPR/Cas9-Mediated Correction of the Sickle Mutation in Human CD34+ cells. Mol. Ther. *24*, 1561–1569.

98. Lomova, A., Clark, D.N., Campo-Fernandez, B., Flores-Bjurström, C., Kaufman, M.L., Fitz-Gibbon, S., Wang, X., Miyahira, E.Y., Brown, D., DeWitt, M.A., et al. (2019). Improving gene editing outcomes in human hematopoietic stem and progenitor cells by temporal control of DNA repair. Stem Cells *37*, 284–294.

99. Park, S.H., Lee, C.M., Dever, D.P., Davis, T.H., Camarena, J., Srifa, W., Zhang, Y., Paikari, A., Chang, A.K., Porteus, M.H., et al. (2019). Highly efficient editing of the β-globin gene in patient-derived hematopoietic stem and progenitor cells to treat sickle cell disease. Nucleic Acids Res. *47*, 7955–7972.

100. Forget, B.G. (1998). Molecular basis of hereditary persistence of fetal hemoglobin. Ann. N Y Acad. Sci. *850*, 38–44.

101. Musallam, K.M., Sankaran, V.G., Cappellini, M.D., Duca, L., Nathan, D.G., and Taher, A.T. (2012). Fetal hemoglobin levels and morbidity in untransfused patients with β-thalassemia intermedia. Blood *119*, 364–367.

102. Sankaran, V.G., Menne, T.F., Xu, J., Akie, T.E., Lettre, G., Van Handel, B., Mikkola, H.K., Hirschhorn, J.N., Cantor, A.B., and Orkin, S.H. (2008). Human fetal hemoglobin expression is regulated by the developmental stage-specific repressor BCL11A. Science *322*, 1839–1842.

103. Gilman, J.G., Mishima, N., Wen, X.J., Stoming, T.A., Lobel, J., and Huisman, T.H. (1988). Distal CCAAT box deletion in the A gamma globin gene of two black adolescents with elevated fetal A gamma globin. Nucleic Acids Res. *16*, 10635–10642.

104. Luc, S., Huang, J., McEldoon, J.L., Somuncular, E., Li, D., Rhodes, C., Mamoor, S., Hou, S., Xu, J., and Orkin, S.H. (2016). Bcl11a deficiency leads to hematopoietic stem cell defects with an aging-like phenotype. Cell Rep. *16*, 3181–3194.

105. Liu, P., Keller, J.R., Ortiz, M., Tessarollo, L., Rachel, R.A., Nakamura, T., Jenkins, N.A., and Copeland, N.G. (2003). Bcl11a is essential for normal lymphoid development. Nat. Immunol. *4*, 525–532.

106. Ippolito, G.C., Dekker, J.D., Wang, Y.H., Lee, B.K., Shaffer, A.L., 3rd, Lin, J., Wall, J.K., Lee, B.S., Staudt, L.M., Liu, Y.J., et al. (2014). Dendritic cell fate is determined by BCL11A. Proc. Natl. Acad. Sci. USA *111*, E998–E1006.

107. Bauer, D.E., Kamran, S.C., Lessard, S., Xu, J., Fujiwara, Y., Lin, C., Shao, Z., Canver, M.C., Smith, E.C., Pinello, L., et al. (2013). An erythroid enhancer of BCL11A subject to genetic variation determines fetal hemoglobin level. Science *342*, 253–257.

108. Wu, Y., Zeng, J., Roscoe, B.P., Liu, P., Yao, Q., Lazzarotto, C.R., Clement, K., Cole, M.A., Luk, K., Baricordi, C., et al. (2019). Highly efficient therapeutic gene editing of human hematopoietic stem cells. Nat. Med. *25*, 776–783.

109. Frangoul, H., Altshuler, D., Cappellini, M.D., Chen, Y.S., Domm, J., Eustace, B.K., Foell, J., de la Fuente, J., Grupp, S., Handgretinger, R., et al. (2021). CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia. N. Engl. J. Med. *384*, 252–260.

110. Neelapu, S.S., Locke, F.L., Bartlett, N.L., Lekakis, L.J., Miklos, D.B., Jacobson, C.A., Braunschweig, I., Oluwole, O.O., Siddiqi, T., Lin, Y., et al. (2017). Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. N. Engl. J. Med. *377*, 2531–2544.

111. Schuster, S.J., Bishop, M.R., Tam, C.S., Waller, E.K., Borchmann, P., McGuirk, J.P., Jäger, U., Jaglowski, S., Andreadis, C., Westin, J.R., et al.; JULIET Investigators (2019). Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. N. Engl. J. Med. *380*, 45–56.

112. Abramson, J.S., Palomba, M.L., Gordon, L.I., Lunning, M.A., Wang, M., Arnason, J., Mehta, A., Purev, E., Maloney, D.G., Andreadis, C., et al. (2020). Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): a multicentre seamless design study. Lancet *396*, 839–852.

113. Wang, M., Munoz, J., Goy, A., Locke, F.L., Jacobson, C.A., Hill, B.T., Timmerman, J.M., Holmes, H., Jaglowski, S., Flinn, I.W., et al. (2020). KTE-X19 CAR T-cell therapy in relapsed or refractory mantle-cell lymphoma. N. Engl. J. Med. *382*, 1331–1342.

114. Munshi, N.C., Anderson, L.D., Jr., Shah, N., Madduri, D., Berdeja, J., Lonial, S., Raje, N., Lin, Y., Siegel, D., Oriol, A., et al. (2021). Idecabtagene vicleucel in relapsed and refractory multiple myeloma. N. Engl. J. Med. *384*, 705–716.

115. Taraseviciute, A., Tkachev, V., Ponce, R., Turtle, C.J., Snyder, J.M., Liggitt, H.D., Myerson, D., Gonzalez-Cuyar, L., Baldessari, A., English, C., et al. (2018). Chimeric antigen receptor T cell-mediated neurotoxicity in nonhuman primates. Cancer Discov. *8*, 750–763.

116. Ehninger, A., Kramer, M., Röllig, C., Thiede, C., Bornhäuser, M., von Bonin, M., Wermke, M., Feldmann, A., Bachmann, M., Ehninger, G., and Oelschlägel, U. (2014). Distribution and levels of cell surface expression of CD33 and CD123 in acute myeloid leukemia. Blood Cancer J. *4*, e218.

117. Taussig, D.C., Pearce, D.J., Simpson, C., Rohatiner, A.Z., Lister, T.A., Kelly, G., Luongo, J.L., Danet-Desnoyers, G.A., and Bonnet, D. (2005). Hematopoietic stem cells express multiple myeloid markers: implications for the origin and targeted therapy of acute myeloid leukemia. Blood *106*, 4086–4092.

118. Brinkman-Van der Linden, E.C., Angata, T., Reynolds, S.A., Powell, L.D., Hedrick, S.M., and Varki, A. (2003). CD33/Siglec-3 binding specificity, expression pattern, and consequences of gene deletion in mice. Mol. Cell. Biol. *23*, 4199–4206.

119. Borot, F., Wang, H., Ma, Y., Jafarov, T., Raza, A., Ali, A.M., and Mukherjee, S. (2019). Gene-edited stem cells enable CD33-directed immune therapy for myeloid malignancies. Proc. Natl. Acad. Sci. USA *116*, 11978–11987.

120. Mendell, J.R., Shilling, C., Leslie, N.D., Flanigan, K.M., al-Dahhak, R., Gastier-Foster, J., Kneile, K., Dunn, D.M., Duval, B., Aoyagi, A., et al. (2012). Evidence-based path to newborn screening for Duchenne muscular dystrophy. Ann. Neurol. *71*, 304–313.

121. Mah, J.K., Korngut, L., Dykeman, J., Day, L., Pringsheim, T., and Jette, N. (2014). A systematic review and meta-analysis on the epidemiology of Duchenne and Becker muscular dystrophy. Neuromuscul. Disord. *24*, 482–491.

122. Hoffman, E.P., Brown, R.H., Jr., and Kunkel, L.M. (1987). Dystrophin: the protein product of the Duchenne muscular dystrophy locus. Cell *51*, 919–928.

123. Duan, D., Goemans, N., Takeda, S., Mercuri, E., and Aartsma-Rus, A. (2021). Duchenne muscular dystrophy. Nat. Rev. Dis. Primers *7*, 13.

124. Bladen, C.L., Salgado, D., Monges, S., Foncuberta, M.E., Kekou, K., Kosma, K., Dawkins, H., Lamont, L., Roy, A.J., Chamova, T., et al. (2015). The TREAT-NMD DMD Global Database: analysis of more than 7,000 Duchenne muscular dystrophy mutations. Hum. Mutat. *36*, 395–402.

125. Aartsma-Rus, A., Ginjaar, I.B., and Bushby, K. (2016). The importance of genetic diagnosis for Duchenne muscular dystrophy. J. Med. Genet. *53*, 145–151.

126. Amoasii, L., Long, C., Li, H., Mireault, A.A., Shelton, J.M., Sanchez-Ortiz, E., McAnally, J.R., Bhattacharyya, S., Schmidt, F., Grimm, D., et al. (2017). Single-cut genome editing restores dystrophin expression in a new mouse model of muscular dystrophy. Sci. Transl. Med. *9*, eaan8081.

127. Kim, K., Ryu, S.M., Kim, S.T., Baek, G., Kim, D., Lim, K., Chung, E., Kim, S., and Kim, J.S. (2017). Highly efficient RNA-guided base editing in mouse embryos. Nat. Biotechnol. *35*, 435–437.

128. Koo, T., Lu-Nguyen, N.B., Malerba, A., Kim, E., Kim, D., Cappellari, O., Cho, H.Y., Dickson, G., Popplewell, L., and Kim, J.S. (2018). Functional rescue of dystrophin deficiency in mice caused by frameshift mutations using Campylobacter jejuni Cas9. Mol. Ther. *26*, 1529–1538.

129. Amoasii, L., Li, H., Zhang, Y., Min, Y.L., Sanchez-Ortiz, E., Shelton, J.M., Long, C., Mireault, A.A., Bhattacharyya, S., McAnally, J.R., et al. (2019). In vivo non-invasive monitoring of dystrophin correction in a new Duchenne muscular dystrophy reporter mouse. Nat. Commun. *10*, 4537.

130. Egorova, T.V., Zotova, E.D., Reshetov, D.A., Polikarpova, A.V., Vassilieva, S.G., Vlodavets, D.V., Gavrilov, A.A., Ulianov, S.V., Buchman, V.L., and Deykin, A.V. (2019). CRISPR/Cas9-generated mouse model of Duchenne muscular dystrophy recapitulating a newly identified large 430 kb deletion in the human *DMD* gene. Dis. Model. Mech. *12*, dmm037655.

131. Min, Y.L., Li, H., Rodriguez-Caycedo, C., Mireault, A.A., Huang, J., Shelton, J.M., McAnally, J.R., Amoasii, L., Mammen, P.P.A., Bassel-Duby, R., and Olson, E.N. (2019). CRISPR-Cas9 corrects Duchenne muscular dystrophy exon 44 deletion mutations in mice and human cells. Sci. Adv. *5*, eaav4324.

132. Min, Y.L., Chemello, F., Li, H., Rodriguez-Caycedo, C., Sanchez-Ortiz, E., Mireault, A.A., McAnally, J.R., Shelton, J.M., Zhang, Y., Bassel-Duby, R., and Olson, E.N. (2020). Correction of three prominent mutations in mouse and human models of Duchenne muscular dystrophy by single-cut genome editing. Mol. Ther. *28*, 2044–2055.

133. Larcher, T., Lafoux, A., Tesson, L., Remy, S., Thepenier, V., François, V., Le Guiner, C., Goubin, H., Dutilleul, M., Guigand, L., et al. (2014). Characterization of dystrophin deficient rats: a new model for Duchenne muscular dystrophy. PLoS ONE *9*, e110371.

134. Nakamura, K., Fujii, W., Tsuboi, M., Tanihata, J., Teramoto, N., Takeuchi, S., Naito, K., Yamanouchi, K., and Nishihara, M. (2014). Generation of muscular dystrophy model rats with a CRISPR/Cas system. Sci. Rep. *4*, 5635.

135. Sui, T., Lau, Y.S., Liu, D., Liu, T., Xu, L., Gao, Y., Lai, L., Li, Z., and Han, R. (2018). A novel rabbit model of Duchenne muscular dystrophy generated by CRISPR/Cas9. Dis. Model. Mech. *11*, dmm032201.

136. Xie, J., Ge, W., Li, N., Liu, Q., Chen, F., Yang, X., Huang, X., Ouyang, Z., Zhang, Q., Zhao, Y., et al. (2019). Efficient base editing for multiple genes and loci in pigs using base editors. Nat. Commun. *10*, 2852.

137. Chen, Y., Zheng, Y., Kang, Y., Yang, W., Niu, Y., Guo, X., Tu, Z., Si, C., Wang, H., Xing, R., et al. (2015). Functional disruption of the dystrophin gene in rhesus monkey using CRISPR/Cas9. Hum. Mol. Genet. *24*, 3764–3774.

138. Yokota, T., Duddy, W., and Partridge, T. (2007). Optimizing exon skipping therapies for DMD. Acta Myol. *26*, 179–184.

139. Godfrey, C., Muses, S., McClorey, G., Wells, K.E., Coursindel, T., Terry, R.L., Betts, C., Hammond, S., O'Donovan, L., Hildyard, J., et al. (2015). How much dystrophin is enough: the physiological consequences of different levels of dystrophin in the mdx mouse. Hum. Mol. Genet. *24*, 4225–4237.

140. van Putten, M., van der Pijl, E.M., Hulsker, M., Verhaart, I.E., Nadarajah, V.D., van der Weerd, L., and Aartsma-Rus, A. (2014). Low dystrophin levels in heart can delay heart failure in mdx mice. J. Mol. Cell. Cardiol. *69*, 17–23.

141. Truong, D.J., Kühner, K., Kühn, R., Werfel, S., Engelhardt, S., Wurst, W., and Ortiz, O. (2015). Development of an intein-mediated split-Cas9 system for gene therapy. Nucleic Acids Res. *43*, 6450–6458.

142. Lagace, T.A., Curtis, D.E., Garuti, R., McNutt, M.C., Park, S.W., Prather, H.B., Anderson, N.N., Ho, Y.K., Hammer, R.E., and Horton, J.D. (2006). Secreted PCSK9 decreases the number of LDL receptors in hepatocytes and in livers of parabiotic mice. J. Clin. Invest. *116*, 2995–3005.

143. Maxwell, K.N., Fisher, E.A., and Breslow, J.L. (2005). Overexpression of PCSK9 accelerates the degradation of the LDLR in a post-endoplasmic reticulum compartment. Proc. Natl. Acad. Sci. USA *102*, 2069–2074.

144. Abifadel, M., Varret, M., Rabès, J.P., Allard, D., Ouguerram, K., Devillers, M., Cruaud, C., Benjannet, S., Wickham, L., Erlich, D., et al. (2003). Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nat. Genet. *34*, 154–156.

145. Cohen, J.C., Boerwinkle, E., Mosley, T.H., Jr., and Hobbs, H.H. (2006). Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. N. Engl. J. Med. *354*, 1264–1272.

146. Cohen, J., Pertsemlidis, A., Kotowski, I.K., Graham, R., Garcia, C.K., and Hobbs, H.H. (2005). Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9. Nat. Genet. *37*, 161–165.

147. Kent, S.T., Rosenson, R.S., Avery, C.L., Chen, Y.I., Correa, A., Cummings, S.R., Cupples, L.A., Cushman, M., Evans, D.S., Gudnason, V., et al. (2017). *PCSK9* Loss-of-Function Variants, Low-Density Lipoprotein Cholesterol, and Risk of Coronary Heart Disease and Stroke: Data From 9 Studies of Blacks and Whites. Circ. Cardiovasc. Genet. *10*, e001632.

148. Sabatine, M.S., Giugliano, R.P., Keech, A.C., Honarpour, N., Wiviott, S.D., Murphy, S.A., Kuder, J.F., Wang, H., Liu, T., Wasserman, S.M., et al.; FOURIER Steering Committee and Investigators (2017). Evolocumab and clinical outcomes in patients with cardiovascular disease. N. Engl. J. Med. *376*, 1713–1722.

149. Robinson, J.G., Farnier, M., Krempf, M., Bergeron, J., Luc, G., Averna, M., Stroes, E.S., Langslet, G., Raal, F.J., El Shahawy, M., et al.; ODYSSEY LONG TERM Investigators (2015). Efficacy and safety of alirocumab in reducing lipids and cardiovascular events. N. Engl. J. Med. *372*, 1489–1499.

150. Breton, C., Clark, P.M., Wang, L., Greig, J.A., and Wilson, J.M. (2020). ITR-Seq, a next-generation sequencing assay, identifies genome-wide DNA editing sites in vivo following adeno-associated viral vector-mediated genome editing. BMC Genomics *21*, 239.

151. Gaudelli, N.M., Lam, D.K., Rees, H.A., Solá-Esteves, N.M., Barrera, L.A., Born, D.A., Edwards, A., Gehrke, J.M., Lee, S.J., Liquori, A.J., et al. (2020). Directed evolution of

adenine base editors with increased activity and therapeutic application. Nat. Biotechnol. *38*, 892–900.

152. Petri, K., Kim, D.Y., Sasaki, K.E., Canver, M.C., Wang, X., Shah, H., Lee, H., Horng, J.E., Clement, K., Iyer, S., et al. (2021). Global-scale CRISPR gene editor specificity profiling by ONE-seq identifies population-specific, variant off-target effects. bioRxiv. https://doi.org/10.1101/2021.04.05.438458.

153. Koblan, L.W., Doman, J.L., Wilson, C., Levy, J.M., Tay, T., Newby, G.A., Maianti, J.P., Raguram, A., and Liu, D.R. (2018). Improving cytidine and adenine base editors by expression optimization and ancestral reconstruction. Nat. Biotechnol. *36*, 843–846.

154. Quinn, J., Musa, A., Kantor, A., McClements, M.E., Cehajic-Kapetanovic, J., MacLaren, R.E., and Xue, K. (2021). Genome-editing strategies for treating human retinal degenerations. Hum. Gene Ther. *32*, 247–259.

155. Berger, W., Kloeckener-Gruissem, B., and Neidhardt, J. (2010). The molecular basis of human retinal and vitreoretinal diseases. Prog. Retin. Eye Res. *29*, 335–375.

156. Russell, S., Bennett, J., Wellman, J.A., Chung, D.C., Yu, Z.F., Tillman, A., Wittes, J., Pappas, J., Elci, O., McCague, S., et al. (2017). Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: a randomised, controlled, open-label, phase 3 trial. Lancet *390*, 849–860.

157. Moshiri, A., Chen, R., Kim, S., Harris, R.A., Li, Y., Raveendran, M., Davis, S., Liang, Q., Pomerantz, O., Wang, J., et al. (2019). A nonhuman primate model of inherited retinal disease. J. Clin. Invest. *129*, 863–874.

158. Koenekoop, R.K. (2004). An overview of Leber congenital amaurosis: a model to understand human retinal development. Surv. Ophthalmol. *49*, 379–398.

159. Stone, E.M. (2007). Leber congenital amaurosis - a model for efficient genetic testing of heterogeneous disorders: LXIV Edward Jackson Memorial Lecture. Am. J. Ophthalmol. *144*, 791–811.

160. Perrault, I., Rozet, J.M., Gerber, S., Ghazi, I., Leowski, C., Ducroq, D., Souied, E., Dufier, J.L., Munnich, A., and Kaplan, J. (1999). Leber congenital amaurosis. Mol. Genet. Metab. *68*, 200–208.

161. den Hollander, A.I., Roepman, R., Koenekoop, R.K., and Cremers, F.P. (2008). Leber congenital amaurosis: genes, proteins and disease mechanisms. Prog. Retin. Eye Res. *27*, 391–419.

162. Chacon-Camacho, O.F., and Zenteno, J.C. (2015). Review and update on the molecular basis of Leber congenital amaurosis. World J. Clin. Cases *3*, 112–124.

163. den Hollander, A.I., Koenekoop, R.K., Yzer, S., Lopez, I., Arends, M.L., Voesenek, K.E., Zonneveld, M.N., Strom, T.M., Meitinger, T., Brunner, H.G., et al. (2006). Mutations in the CEP290 (NPHP6) gene are a frequent cause of Leber congenital amaurosis. Am. J. Hum. Genet. *79*, 556–561.

164. Geller, A.M., and Sieving, P.A. (1993). Assessment of foveal cone photoreceptors in Stargardt's macular dystrophy using a small dot detection task. Vision Res. *33*, 1509–1524.

165. Payne, A.M., Morris, A.G., Downes, S.M., Johnson, S., Bird, A.C., Moore, A.T., Bhattacharya, S.S., and Hunt, D.M. (2001). Clustering and frequency of mutations in the retinal guanylate cyclase (GUCY2D) gene in patients with dominant cone-rod dystrophies. J. Med. Genet. *38*, 611–614.

166. Kitiratschky, V.B., Wilke, R., Renner, A.B., Kellner, U., Vadalà, M., Birch, D.G., Wissinger, B., Zrenner, E., and Kohl, S. (2008). Mutation analysis identifies GUCY2D as the major gene responsible for autosomal dominant progressive cone degeneration. Invest. Ophthalmol. Vis. Sci. *49*, 5015–5023.

167. Gregory-Evans, K., Kelsell, R.E., Gregory-Evans, C.Y., Downes, S.M., Fitzke, F.W., Holder, G.E., Simunovic, M., Mollon, J.D., Taylor, R., Hunt, D.M., et al. (2000). Autosomal dominant cone-rod retinal dystrophy (CORD6) from heterozygous mutation of GUCY2D, which encodes retinal guanylate cyclase. Ophthalmology *107*, 55–61.

168. Harding, J., Roberts, R.M., and Mirochnitchenko, O. (2013). Large animal models for stem cell therapy. Stem Cell Res. Ther. *4*, 23.

169. Lee, W.G., Demirci, U., and Khademhosseini, A. (2009). Microscale electroporation: challenges and perspectives for clinical applications. Integr. Biol. *1*, 242–251.

170. Peterson, C.W., and Kiem, H.P. (2019). Lessons from London and Berlin: Designing a scalable gene therapy approach for HIV cure. Cell Stem Cell *24*, 685–687.

171. Wang, H., Gil, S., Li, G., Doering, C., Ng, P., Roffler, S., Kiem, H.P., and Lieber, A.L. (2021). In vivo HSC gene therapy with high-level, erythroid-specific expression of a secreted SARS-CoV-2 decoy receptor (abstract). Mol. Ther. *29* (*Suppl 1*), 91–92.

172. Li, C., Wang, H., Georgakopoulou, A., Gil, S., Yannaki, E., and Lieber, A. (2021). In Vivo HSC Gene Therapy Using a Bi-modular HDAd5/35++ Vector Cures Sickle Cell Disease in a Mouse Model. Mol. Ther. *29*, 822–837.

173. Liu, S., Cheng, Q., Wei, T., Yu, X., Johnson, L.T., Farbiak, L., and Siegwart, D.J. (2021). Membrane-destabilizing ionizable phospholipids for organ-selective mRNA delivery and CRISPR-Cas gene editing. Nat. Mater. *20*, 701–710.

174. Li, C., and Lieber, A. (2019). Adenovirus vectors in hematopoietic stem cell genome editing. FEBS Lett. *593*, 3623–3648.

175. Rosenblum, D., Gutkin, A., Kedmi, R., Ramishetti, S., Veiga, N., Jacobi, A.M., Schubert, M.S., Friedmann-Morvinski, D., Cohen, Z.R., Behlke, M.A., et al. (2020). CRISPR-Cas9 genome editing using targeted lipid nanoparticles for cancer therapy. Sci. Adv. *6*, eabc9450.

176. Song, C.Q., Jiang, T., Richter, M., Rhym, L.H., Koblan, L.W., Zafra, M.P., Schatoff, E.M., Doman, J.L., Cao, Y., Dow, L.E., et al. (2020). Adenine base editing in an adult mouse model of tyrosinaemia. Nat. Biomed. Eng. *4*, 125–130.