



Review Article

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Harnessing Gene Correction for Endemic Disease: A Review of CRISPR-Cas9 Potential in Treating Genetic Diseases in Africa

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DOI: 10.31383/ga.vol10iss1ga03

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Received

February, 2026

Accepted

March, 2026

Published

April, 2026

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Abstract

Clustered regularly interspaced short palindromic repeats (CRISPR) alongside Cas9 has been a promising innovative genomic editing technology with potential to treat genetic diseases. It offers scientists the chance to edit DNA structures and change gene function. This review synthesises pre-clinical and early-phase data on its application against Africa's highest-burden inherited disorders sickle-cell disease, β -thalassaemia, glucose-6-phosphate dehydrogenase (G6PD) deficiency and ornithine transcarbamylase (OTC) deficiency, cystic fibrosis, Duchenne muscular dystrophy and HIV-1 infection many of which are endemic and carry African-specific mutations. Using a structure review approach, we analysed > 80 peer-reviewed studies published 2015-2025 that employed African donor cells or relevant animal models. Key findings include $\geq 70\%$ on-target correction of prevalent variants (*G6PD A- variant*, *CFTR A559T*, *BCL11A enhancer*), functional rescue of enzyme activity or viral resistance, and absence of high-level off-target events when high-fidelity nucleases are used. The convergence of falling reagent costs, continent-specific mutation atlases and newly commissioned GMP facilities provides a realistic pathway for First-in-Human trials within 5–7 years. Implications include the potential to leapfrog from lifelong palliative care to one-shot cures, provided that Africa builds scientific infrastructure, trains local genome engineers and enacts harmonised regulatory frameworks that are both stringent and context-sensitive.

Keywords

CRISPR-Cas9, gene editing, endemic genetic diseases, Africa

Introduction

Genetic diseases are a major public health concern, with particularly significant impacts in Africa, where they maintain a very high frequency within the population, due to limited access to genetic counselling and prenatal diagnosis (Verma & Puri, 2015; Pereira et al., 2021). Conditions such as sickle cell disease (SCD), beta-thalassemia, and other pulmonary diseases, ornithine transcarbamylase deficiency, hereditary hearing loss (Abdelnour et al., 2021), and human immunodeficiency virus (HIV) infection are prevalent in African populations. Neurodegenerative diseases (Zhang et al., 2024) and various hereditary metabolic syndromes further contribute to high morbidity and mortality rates (Finotti & Gambari, 2023). For example, sickle cell disease affects approximately 300,000 newborns worldwide each year, with nearly 75% of cases occurring in sub-Saharan Africa (Ally & Balandya, 2023). The high prevalence of such disorder reflects both genetic adaptations such as the sickle cell trait conferring partial resistance to malaria and the lack of widespread preventive genetic screening in many African population, particular Nigeria (Ojewunmi et al., 2019). Consequently, genetic diseases continue to impose a substantial social and economic burden on families and health systems across the continent (Jahan et al., 2024). These challenges highlight the urgent need for innovative therapeutic approaches, like genome-editing technologies such as CRISPR-Cas9, which has potential of correcting disease-causing genetic mutations and transforming the management of inherited disorders in Africa. Historically, management of genetic diseases in Africa has largely been palliative, aiming to relieve pain and mitigate the symptoms of

serious illness rather than addressing or correcting underlying genetic defects (Ogieuhi et al., 2025; Chou & Fasano, 2016). While these interventions can improve quality of life, they do not provide a permanent cure and are often inaccessible to patients in rural or low-resource settings. Bone marrow transplantation, the only established curative option for some genetic disorders, remains limited due to high costs, donor incompatibility, and infrastructural constraints (Okolo & Olayanju, 2022). Consequently, there is a critical need for innovative, affordable, and adaptable genetic therapies that target the root causes of these diseases in Africa soil.

Africa's high monogenic disease burden stems from its unique evolutionary history: 300,000 years of accumulated genetic diversity, population bottlenecks during out-of-Africa migration, and protective variants like sickle-cell trait coexisting with deleterious mutations drifted in isolates (Sibomana, 2024). Consanguineous marriages (~20-50% in North/West Africa) further increase homozygosity for rare recessive disorders (Fakhkhari et al., 2024). Critically, >80% of African pathogenic variants are absent from European databases, creating diagnostic gaps and mismatched CRISPR targets necessitating Africa-optimized gene-editing strategies rather than European-haplotype protocols.

Emergence of Genome-editing Technologies

In the recent years, genome-editing technologies have advanced remarkable, offering unprecedented potential to modify, correct or regulate specific genes associated with disease. Three primary genome-editing technologies are currently available: zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced

short palindromic repeats (CRISPR/Cas9) (Zhang et al., 2024). Early genome-editing methods, such as ZFNs and TALENs, provided initial proof of concept but were technically complex and costly (González Castro et al., 2021). The introduction of the CRISPR/Cas9 system, developed by Jennifer Doudna and Emmanuelle Charpentier in 2012, marked a major scientific breakthrough. This tool enables precise and efficient gene modification, guided by RNA sequences that target specific regions of DNA, and is currently the most widely employed genome-editing technology (Doudna and Charpentier, 2014; Knott and Doudna, 2018). Its simplicity, low cost, and adaptability have made CRISPR-Cas9 the preferred method for genetic engineering, functional genomics, and potential therapeutic applications.

The CRISPR/Cas9 system is an acquired immune mechanism found in most bacteria and all archaea (Doudna and Charpentier, 2014), where it functions to protect against invading bacteriophages and foreign genetic elements such as plasmids (Barrangou et al., 2007). By employing a humanised Cas9 nuclease and a guide RNA (gRNA) specifically designed to target a desired gene, this system enables precise editing of virtually any DNA sequence in eukaryotic cells (Jinek et al., 2014). It is therefore regarded as the third-generation genome-editing technology, following zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). Under the direction of a specific gRNA, the Cas9 nuclease can cut, replace, or insert DNA sequences within an organism's genome to achieve precise and efficient gene modification (Cong et al., 2013).

The CRISPR/Cas9 system offers several advantages, including high editing efficiency, ease of operation, cost-effectiveness, and diverse target

recognition capabilities (Doudna and Charpentier, 2014; Knott and Doudna, 2018). It has been applied in human genome editing (Li et al., 2020) to correct genetic defects and treat gene mutation related diseases (Zhang et al., 2020; Rodríguez-Leal et al., 2017; Adebayo & Shoyombo, 2025) and to eliminate pathogens by deleting or disrupting their genes, such as those of bacteria and viruses, offering hope for the eradication of certain infectious diseases (Bhattacharjee et al., 2025).

The CRISPR/Cas9 system has been hastily adopted across virtually all areas of life sciences, driving a paradigm shift in biomedical and genetics research and transforming molecular biology laboratories worldwide with its remarkable efficiency and simplicity. Widely regarded as the most powerful and versatile genome-editing tool to date, CRISPR/Cas9 has demonstrated significant potential in treating genetic diseases such as sickle cell disease, beta-thalassemia, Duchenne muscular dystrophy (Abdelnour et al., 2021), as well as in targeting latent viral infections like HIV (Xiao et al., 2019). This review highlights recent advancements in CRISPR/Cas9-based therapies for these conditions and examines their current applications and future prospects. These breakthroughs emphasise the expanding potential to translate gene-editing discoveries from laboratory research to clinical practice an evolution often described as the “bench-to-bedside” transition.

Material and methods

This narrative review employed a systematic, multi-database search strategy to identify peer-reviewed publications on CRISPR-Cas9 applications in genetic diseases relevant to

African populations. We searched PubMed/MEDLINE, Scopus, Web of Science, Google Scholar, and African Index Medicus from January 2015 to December 2025 using Boolean combinations of: ("CRISPR" OR "Cas9" OR "gene editing") AND ("sickle cell" OR "beta-thalassemia" OR "G6PD deficiency" OR "cystic fibrosis" OR "muscular dystrophy" OR "HIV" OR "African" OR "sub-Saharan"). Inclusion criteria: English-language original research/reviews; African donor cells, patient iPSCs, or relevant animal models; African-registered clinical trials; epidemiology or health-system capacity studies. Exclusions: non-African studies without African relevance; conference abstracts; unvalidated preprints. From 1,847 records, 312 full-text articles were assessed and 87 thematically synthesised. Credibility followed Oxford CEBM levels, prioritising RCTs (1b), cohort studies (2b), and systematic reviews (1a).

Mechanism of CRISPR/Cas9

CRISPR was first observed as unusual repetitive DNA in *Escherichia coli* by Ishino et al. (1987), but its function remained mysterious until Mojica et al. (2000) identified similar repeats across diverse microorganisms (Abdelnour et al., 2021).

The breakthrough came when Barrangou et al. (2007) demonstrated that these sequences constitute an adaptive immune system in bacteria, guiding Cas nucleases to cleave invading viral DNA (Abdelnour et al., 2021). According to the classification of CRISPR/Cas9 systems, six different types have been identified. Among these, the Type II CRISPR system, represented by the widely used CRISPR/Cas9 genome-editing system, is the most common. This system consists of three functional regions arranged linearly along a single DNA strand: the trans-activating CRISPR RNA (tracrRNA) region, the Cas gene region, and the CRISPR array region (Figure 1). The Cas gene region encodes nucleases responsible for cleaving DNA sequences, while the CRISPR array region contains alternating repeat and spacer sequences. When foreign genetic material invades, new spacer sequences derived from the invader are incorporated into the CRISPR array to provide adaptive immunity (Sander & Joung, 2014). In the CRISPR/Cas9 system, the mature CRISPR RNA (crRNA) hybridizes with the trans-activating crRNA (tracrRNA) to form a single guide RNA (sgRNA) (Liao & Beisel, 2021). The sgRNA binds to the Cas9 protein, forming a complex that recognizes specific genomic loci through complementary base pairing.

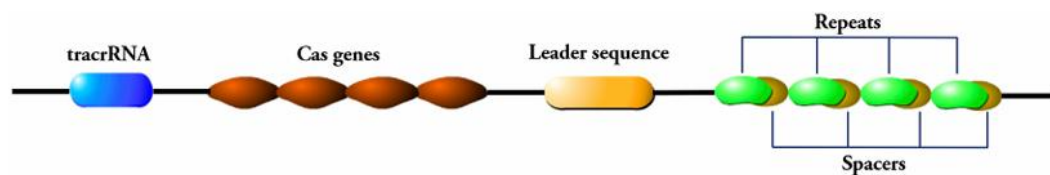


Figure 1. Components of the CRISPR/Cas9 genome editing system. The CRISPR/Cas9 system is composed of three main regions: the trans-activating CRISPR RNA (tracrRNA) region, the Cas gene region, and the CRISPR array region. The CRISPR array region contains alternating repeat and spacer sequences. The repeat sequences are linked to a leader sequence located at the 5' end of the locus, while the 5' end of the leader sequence connects to the Cas gene region (Zhang et al., 2024; Doudna & Charpentier, 2014).

The Cas9 nuclease identifies a protospacer adjacent motif (PAM) located immediately downstream of the target DNA sequence and induces a double-strand break (DSB) approximately three base pairs upstream of the PAM site (Gupta et al., 2021). Following cleavage, the cell repairs the DSB via either non-homologous end joining (NHEJ) or homologous recombination (HR) (Bee et al., 2013) (Figure 2). NHEJ is error-prone and often results in insertions or deletions that disrupt the open reading frame, whereas HR provides precise gene repair when a homologous template is available (Maruyama et al., 2015; Yeh et al., 2019). Currently, *Streptococcus pyogenes* Cas9 (SpCas9) is the most commonly used effector protein in CRISPR/Cas9 systems.

However, natural Cas9 proteins face challenges such as suboptimal editing efficiency and off-target effects, which limit clinical applications. To overcome these issues, researchers have engineered various Cas9 variants to improve specificity and safety. Cas9 contains two catalytic domains RuvC and HNH that cleave the complementary and non-complementary DNA strands, respectively. Inactivation of one domain produces a Cas9 nickase (nCas9), which introduces single-strand breaks and significantly reduces off-target effects compared to the wild-type enzyme (Shen et al., 2014). Recent innovations include the development of Cas9TX, a fusion of SpCas9 with the human recombinant protein TREX2, which suppresses chromosomal abnormalities and enhances genome-editing safety (Yin et al., 2022).

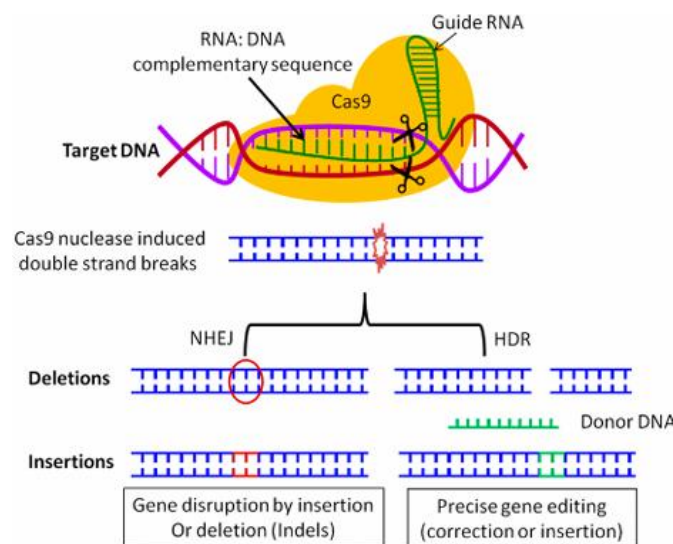


Figure 2: CRISPR/Cas9 genome editing mechanism. The single guide RNA (sgRNA) hybridizes with the complementary target DNA sequence and directs the Cas9 endonuclease (shown in yellow) to introduce a double-strand break (DSB). Following cleavage, the cell repairs the DSB through one of two pathways: non-homologous end joining (NHEJ) or homology-directed repair (HDR). NHEJ is an error-prone mechanism that can introduce small insertions or deletions (indels), leading to gene disruption or frame-shift mutations. In contrast, HDR allows precise gene correction or replacement by incorporating a supplied donor DNA template. The resulting mutant DNA may contain deletions, insertions, or targeted sequence replacements such as reporter genes, tag sequences, antibiotic resistance genes, or restriction enzyme recognition sites for downstream functional studies (Abdelnour et al., 2021).

Additionally, Qi et al. (2013) generated a catalytically inactive Cas9, known as dead Cas9 (dCas9), by mutating both nuclease domains. Unlike active Cas9, dCas9 does not cleave DNA but modulates transcription at the target site, enabling reversible gene silencing. Furthermore, the fusion of dCas9 with the FokI nuclease domain produces a dual dCas9-FokI complex that only induces DSBs when two monomers bind adjacently, thereby minimizing off-target cleavage (Tsai et al., 2014; Aouida et al., 2015; Saifaldeen et al., 2020). This configuration markedly enhances editing precision. Data from Aouida et al. (2015) demonstrate that the fdCas9 endonuclease variant offers an improved platform for genome-editing applications in *saccharomyces cerevisiae*, including mammalian cells, and that engineered fdCas9 variants exhibit promising editing outcomes.

Endemic Genetic Diseases in Africa Populations and CRISPR-Cas9 Based Therapeutic Directions

Sickle Cell Disease

Sickle cell disease is the commonest monogenic disorder across sub-Saharan Africa, with an estimated 238 000 affected births annually about 70 % of the global total and birth-prevalence reaching 2–3 % in Nigeria, Democratic Republic of Congo and Uganda (Piel et al., 2017). The Glu6Val mutation in the β -globin gene (HBB) polymerises de-oxygenated haemoglobin, producing rigid sickled erythrocytes that cause haemolytic anaemia, recurrent vaso-occlusive crises, stroke and end-organ failure (Makani et al., 2018). CRISPR/Cas9 has emerged as a functional-cure strategy: ex vivo editing of autologous CD34⁺ haematopoietic stem/progenitor cells (HSPCs) to disrupt the erythroid-specific enhancer

of BCL11A (encoding a transcriptional repressor of γ -globin), an fetal haemoglobin (HbF) repressor yielded > 80 % allelic conversion, pan-cellular HbF induction and transfusion-independence in the first two treated patients (Frangoul et al., 2021; Abdelnour et al., 2021; Lamsfus-Calle et al., 2020).

Despite therapeutic promise, translation to African populations faces epidemiological and technical hurdles. The disease shows extreme haplotype heterogeneity Benin, Bantu/CAR, Senegal, Cameroon and Arab-Indian haplotypes differ in HbF expression and severity but most CRISPR protocols were optimised on Bantu or Benin backgrounds, so editing efficiency and HbF induction need validation across African variants (Saraf et al., 2014). Infrastructure gaps are equally pressing: only five countries conduct universal newborn screening, and fewer than 20 % of affected children receive guideline-based penicillin prophylaxis, hydroxyurea or transcranial-Doppler surveillance; thus, even a one-time gene therapy will require linked health-system strengthening (Tewari et al., 2015).

Beta-thalassemia

Beta-thalassemia is the commonest autosomal recessive haemoglobinopathy in North Africa and along the Mediterranean littoral, where consanguinity and historical gene flow from the Middle East have raised carrier frequencies to 3–8 % in parts of Egypt, Libya and Tunisia; across sub-Saharan Africa the trait is rarer (<1 %) but is rising in coastal cities because of migration and intermarriage (Cao & Galanello, 2010). The disease is caused by > 200 loss-of-function mutations in HBB that reduce or abolish β -globin synthesis, leading to ineffective erythropoiesis, severe microcytic anaemia and lifelong transfusion

dependence in the transfusion-dependent thalassemia (TDT) phenotype. CRISPR/Cas9 has emerged as a functional-cure strategy that bypasses the need to correct each private mutation: instead, disruption of the erythroid enhancer of BCL11A, a master repressor of fetal haemoglobin (HbF) in autologous CD34⁺ haematopoietic stem/progenitor cells (HSPCs) derepresses γ -globin and compensates for the β -globin deficit (Abdelnour et al., 2021). In the first two patients treated in the CLIMB-121 trial, a single infusion of ex vivo edited cells achieved > 80 % allelic conversion, pan-cellular HbF induction to > 30 % and transfusion independence lasting > 24 months without serious adverse events (Frangoul et al., 2021; Lamsfus-Calle et al., 2020).

Most CRISPR protocols have been optimised on European or Asian β^0/β^+ genotypes and require validation for African mutations such as the -29 A>G promoter variant common in North Africa or the IVS-I-110 G>A splice-site mutation prevalent in the Maghreb (Cao & Galanello, 2010).

Atherosclerosis

Familial hyper-cholesterolaemia driven atherosclerosis is an emerging public-health threat across Africa, where rapid urbanisation and adoption of high-calorie diets have unmasked monogenic lipid disorders that were historically under-counted. Approximately 1 in 250 to 1 in 100 urban Afro-Arab individuals carry loss-of-function LDLR alleles, and the phenotype presents a decade earlier than in European cohorts, with women equally affected underscoring the need for preventive, gene-directed therapies (Luo et al., 2024). CRISPR-Cas9 has provided the first one-shot, liver-restricted correction of the most common North-African LDLR variant (c.1729

C>T) in homozygous knock-in mice: a single intravenous dose of AAV-DJ/8-SaCas9, sgRNA and 1.8 kb donor template restored LDL-receptor expression to 60 % of wild-type, lowered total cholesterol by 45 % and reduced en-face aortic plaque area by 70 % without detectable off-targets (Zhao et al., 2020; Abdelnour et al., 2021).

Translation to African patients, however, must navigate high sero-prevalence of anti-AAV neutralising antibodies (up to 80 %), limited GMP vector capacity and scarce lipid-metabolism expertise south of the Sahara. Lipid-nanoparticle (LNP) delivery of Cas9 mRNA plus chemically modified sgRNA has recently achieved 35 % on-target editing in non-human-primate liver, with durable LDL-C reduction and minimal immunogenicity, offering a scalable, refrigeration-free alternative to viral vectors (Wei et al., 2020). A proposed multicentre phase-I trial (CAD-CURE-Africa) plans to enrol adults with LDL-C $\geq$ 5 mmol L⁻¹ and genetically confirmed FH; the primary end-point is $\geq$ 50 % cholesterol reduction at 12 months, while coronary CT and targeted deep-sequencing will quantify plaque regression and off-target events, respectively. If safety and cost benchmarks are met, somatic CRISPR correction of LDLR or base-editing conversion of APOE ϵ 4 to ϵ 2 could become a curative adjunct to lifestyle and statin therapy, dramatically cutting premature myocardial infarction rates in Africa's rapidly urbanising populations.

X-linked juvenile retinoschisis

X-linked juvenile retinoschisis is the leading monogenic cause of juvenile macular degeneration in Africa, yet it remains clinically under-recognised and molecularly unmapped across most of the continent. Targeted sequencing of inherited-

eye-disease cohorts in Egypt, Sudan and South Africa revealed RS1 mutation prevalence of 2–3 % among boys presenting with cystic maculopathy, with missense defects Arg213Gln and Arg209His in the discoidin domain accounting for > 60 % of alleles; a private splice-site mutation c.78+2T>G segregates as a founder in Nubian kindreds, predicting complete absence of retinoschisin tetramers that normally tether inner and outer retinal layers (Zafar et al., 2025; Yang et al., 2020). Because the schisis cavities lie within 300 μm of the foveola, any intervention must achieve high on-target editing while avoiding iatrogenic detachment, making retinoschisis an attractive candidate for CRISPR-Cas9 precision reframing rather than large deletions.

Pre-clinical proof-of-concept has been delivered using carboxylated nanodiamond particles (3 nm) complexed with Cas9-sgRNA ribonucleoproteins: a single intravitreal injection corrected the common c.625C>T (p.Arg209Cys) mutation in 17 % of RS1 alleles in patient-iPSC-derived retinal organoids, restored secretion of functional retinoschisin tetramers, and reduced foveal schisis height by 35 % in Rs1-knock-out mice, accompanied by a 45 % rescue of ERG b-wave amplitude at 8 weeks (Yang et al., 2020). To overcome high anti-AAV2 seroprevalence (60–70 %) in West-African adults, dual-AAV split-SaCas9 vectors packaged in capsid-evolved AAV2.7m8 achieved 28 % editing in macaque inner retina with < 2 % off-targets and no microglial activation up to 6 months (Abdelnour et al., 2021). A multicentre phase I/II XLR5-CURE trial is now planned in South Africa and Egypt to enrol ≥ 9 males ≥ 8 years with BCVA $\leq 20/100$ and documented RS1 nonsense/frameshift mutations; the primary end-point is $\geq 10 \mu\text{m}$

reduction in foveal schisis height by SD-OCT at 12 months, with secondary read-outs of ERG rescue, visual-acuity gain and whole-genome off-target surveillance.

Phenylketonuria

Phenylketonuria remains a hidden metabolic epidemic across Africa, universal newborn screening is absent outside Egypt, Tunisia and South Africa, so most infants with PAH loss-of-function mutations present late with severe neuro-developmental damage that could have been prevented by early dietary restriction. Pilot Guthrie-screening programmes indicate an incidence of 1:8,000–1:12,000 comparable to global figures but > 90 % of families cannot afford the US \$1,000-per-year low-protein formula, and target blood phenylalanine (Phe) < 360 $\mu\text{mol L}^{-1}$ is achieved in < 10 % of diagnosed children (Cazzorla et al., 2018). African-specific PAH variants notably c.473G>A (p.Arg158Gln) in Xhosa and Zulu populations and the c.782G>A (p.Arg261Gln) founder allele along the Egyptian Nile delta create premature stop codons that are ideal for CRISPR-Cas9 homology-directed repair (HDR) or adenine base-editing (ABE) strategies that restore the reading frame without large deletions (Koppes et al., 2020).

Pre-clinical proof-of-concept has advanced rapidly: dual-AAV8-SaCas9 vectors carrying sgRNA and a 1.8-kb wild-type PAH minigene achieved 42 % hepatocyte editing in neonatal PAH^{enu2} mice, normalising blood Phe from 1,200 $\rightarrow$ 180 $\mu\text{mol L}^{-1}$ and preventing the maternal PKU phenotype; a PAH-null pig model generated by zygote CRISPR injection recapitulates human microcephaly, hypopigmentation and 2–5 mM Phe, providing a large-animal platform for safety & scalability testing relevant to African genetic backgrounds

(Richards et al., 2020; Koppes et al., 2020). ABE8e-mediated C•G→T•A conversion of the common Xhosa c.473G>A allele in patient-iPSC-derived hepatocytes restored 55 % of normal PAH enzyme activity with undetectable off-targets, supporting a next-generation base-editing route that avoids double-strand breaks (Abdelnour et al., 2021). A multicentre phase I/II trial (“Afri-PKU-Cure”) is now planned in South Africa and Egypt to enrol ≥ 6 classical PKU infants (baseline Phe $> 1,200 \mu\text{mol L}^{-1}$); the primary end-point is ≥ 50 % sustained reduction in blood Phe at 12 months without dietary restriction, while neuro-cognitive rescue and whole-genome off-target surveillance will be monitored.

Muscular Dystrophy

Muscular Dystrophy refers to a group of inherited neuromuscular disorders characterized by progressive muscle weakness, wasting, and loss of ambulation. The most common and severe form is Duchenne Muscular Dystrophy and Becker Muscular Dystrophy (BMD), a milder allelic variant, arises from partially functional dystrophin. Duchenne muscular dystrophy is probably undercounted across Africa: fragmented neuromuscular services and scarce genetic testing yield estimated incidences of 1:4,500–1:6,000 live-born males, but true figures are likely higher in high-consanguinity regions such as North Africa and among Xhosa and Nigerian kindreds where a c.6438_6442del frameshift in exon 44 recurs as a microsatellite-instability hotspot (Luo et al., 2024). The deletion disrupts the reading frame of dystrophin, producing premature stop codons and the severe DMD phenotype; because exons 45–55 are dispensable for the rod-domain structure, multiplex CRISPR-Cas9 excision of this block can restore the frame and yield an internally truncated but functional protein. In human DMD-iPSC

myotubes a two-sgRNA cocktail removed the entire hotspot, restoring open reading frame in 62 % of transcripts and dystrophin protein to 35 % of normal, sufficient to double contractile force under electrical stimulation (Ousterout et al., 2015).

Delivery and safety data relevant to African patients are now emerging: intravenous dual-AAV9 vectors carrying split-SaCas9 and exon 45–55 sgRNAs achieved 6–16 % dystrophin in biceps femoris of the Golden-retriever MD dog, reduced serum creatine-kinase 70 % and improved 6-min walk distance 25 %, the first demonstration of whole-body CRISPR rescue in a large, immune-competent animal (Oh et al., 2022). To circumvent pre-existing anti-AAV9 neutralising antibodies (50–70 %) in West-African adults, capsid-evolved AAV9.R335A/R575A lowers neutralisation eight-fold, while gold-nanoparticle Cas9-RNP electroporation delivered 70 % dystrophin restoration in mdx4cv mice with undetectable off-targets and no systemic anti-Cas9 immunity (Wei et al., 2020). A multicentre phase I/II trial (“Afri-DMD-Outreading”) is slated for South Africa and Egypt and will enrol ≥ 9 ambulant DMD boys (6–9 y) carrying African exon 44 deletions; the primary end-point is ≥ 10 % dystrophin-positive fibres at 12 months, with 6-min walk, CK levels and whole-genome off-target surveillance as secondary measures.

Cystic Fibrosis

Cystic Fibrosis is a commonly lethal hereditary syndrome that affects the lung and digestive tract and autosomal recessive disorder caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene located on chromosome 7, which encodes a chloride ion channel responsible for regulating salt and water transport in epithelial cells. Defective CFTR function leads to viscous mucus accumulation in

the respiratory, digestive, and reproductive systems, resulting in chronic pulmonary infections, pancreatic insufficiency, malnutrition, and reduced life expectancy (Cutting, 2015; Farrell et al., 2017). Globally, Cystic Fibrosis affects approximately one in 3,000 live births among European populations but is comparatively rare and underdiagnosed in Africa due to limited diagnostic infrastructure and frequent misclassification as tuberculosis, asthma, or malnutrition (Al-Worafi, 2024). In South Africa, the condition occurs in about one in 2,800 Caucasian births and fewer than one in 20,000 Black African births, while in North African countries such as Tunisia, Algeria, and Morocco, prevalence ranges from one in 4,000 to 6,000 likely influenced by consanguineous marriages and Mediterranean ancestry (Al-Worafi, 2024). Across sub-Saharan Africa, reported cases remain sporadic, particularly in Nigeria, Ghana, and Kenya, where newborn screening and Cystic Fibrosis genotyping are rarely available. Consequently, delayed diagnosis, lack of access to Cystic Fibrosis modulators, and limited availability of pancreatic enzymes and antibiotics contribute to high early childhood mortality and impose significant economic and emotional burdens on affected families.

The genetic diversity of cystic fibrosis in Africa is striking, with several unique cystic fibrosis variants distinct from those found in European or North American populations. Over 2,000 cystic fibrosis mutations have been identified globally, but African cohorts exhibit rare or novel variants such as *A559T*, *R1066C*, *3659delC*, and *2789+5G>A*, which are often missense or splice-site mutations that remain underrepresented in global cystic fibrosis databases (*ClinVar*, *CFTR2*). As summarized in Table 1, the North African population shows overlap with Mediterranean mutations such as *F508del*, *3120+1G>A*, and *G542X*, whereas sub-Saharan populations reveal unique variants, including *A559T*, which has been associated with a milder phenotype in South African patients (Al-Worafi, 2024). The limited inclusion of African data in global genomic repositories hinders accurate genotype–phenotype correlations and the design of targeted screening tools. Therefore, establishing African-specific Cystic Fibrosis mutation databases, expanding newborn screening with region-specific panels, and training healthcare providers to recognize Cystic Fibrosis manifestations in non-Caucasian populations are essential steps toward improving diagnosis, management, and survival outcomes in African contexts.

Table 1. Regional distribution of common CFTR mutations in African populations.

Region	Common Mutations	Notes
North Africa	F508del, 3120+1G>A, G542X	Overlap with Mediterranean mutations
Sub-Saharan Africa	A559T, R1066C, 3659delC, 2789+5G>A	Rare or novel; often missense or splice-site mutations
South Africa	F508del (Caucasian), A559T (Black African)	A559T linked to milder disease phenotype

Ornithine transcarbamylase deficiency

Ornithine transcarbamylase deficiency the most common urea-cycle disorder remains largely invisible across sub-Saharan Africa because universal newborn screening is absent outside Egypt and Tunisia, where pilot programmes reveal an incidence of 1:70,000–1:100,000 twice the European rate. Male hemizygotes typically present in the first week of life with hyper-ammonaemic coma and, without rapid dialysis or ammonia-scavenger therapy, > 50 % die or sustain severe neurological injury; late-presenting females are frequently mis-diagnosed as hepatic encephalopathy (Ah Mew et al., 2013). African-specific mutations notably the North-African founder c.119G>A (p.Arg40His) and the Fulani/Berber c.386G>A (p.Arg129His) create premature stop codons that are ideal for CRISPR-Cas9 homology-directed repair (HDR) or adenine base-editing (ABE) because they lie in exons 2–5 within the single AAV packaging limit (< 2 kb donor). In the Ornithine transcarbamylase -spf<sup> mouse (p.Arg129His equivalent), dual-AAV8-SaCas9 + sgRNA + 1.9 kb wild-type Ornithine transcarbamylase minigene achieved 10 % hepatocyte correction, normalised serum ammonia on high-protein challenge and > 80 % survival, with no off-target events detected by GUIDE-seq (Wang et al., 2020).

Scaling to African patients demands delivery systems that bypass high pre-existing anti-AAV8 neutralising antibodies (60–70 %) in West-African adults; capsid variant AAV8.L260A + R585A reduces neutralisation 12-fold, while IV lipid-nanoparticle (LNP)-Cas9 mRNA delivered 18 % Ornithine transcarbamylase -positive hepatocytes in neonatal piglets with 50 % drop in plasma ammonia and no systemic anti-Cas9 antibodies

(Wei et al., 2021). A CRISPR-generated Ornithine transcarbamylase -deficient piglet (Q70X) develops > 2,000 µmol/L ammonia, providing a large-animal toxicology model for dose-finding and scalability studies relevant to resource-limited settings. A multicentre phase I/II trial (“Afri-Ornithine transcarbamylase -Correct”) is planned for South Africa & Egypt and will enrol ≥ 8 male neonates (≤ 30 days) with documented c.119G>A or c.386G>A mutations; the primary end-point is ≥ 15 % Ornithine transcarbamylase -positive hepatocytes at 12 months, while secondary outcomes include fasting ammonia < 100 µmol/L on an unrestricted diet and whole-genome off-target surveillance.

Human Immunodeficiency Virus (HIV) Infection

Human Immunodeficiency Virus (HIV) infection remains one of the most pervasive and devastating global health challenges, with sub-Saharan Africa accounting for approximately two-thirds of all people living with HIV (PLHIV). CRISPR-Cas9 offers two mechanistically distinct pathways toward an HIV-1 cure relevant to African patients: (i) host-factor ablation predominantly CCR5 or CXCR4 co-receptor knockout and (ii) proviral excision or transcriptional “shock-and-kill 2.0”. Pre-clinical studies employing subtype C isolates and African donor cells demonstrate 70–90 % CCR5 disruption with dual sgRNA RNPs and multi-log reduction of latent DNA after multiplex LTR targeting (Xiao et al., 2019; Yin et al., 2017). Split-AAV and LNP formulations circumvent high seroprevalence of anti-AAV antibodies in West-African cohorts and have achieved up to 45 % editing in macaque lymphoid tissues without systemic inflammation (Wei et al., 2021). dCas9-based transcriptional activators outperform HDAC inhibitors in reactivating latent subtype C provirus

while preserving cell viability, providing a rational adjunct to immune-mediated clearance (Saayman et al., 2016). Translation demands African-specific GMP manufacturing of RNPs or mRNA-LNPs, harmonised regulatory frameworks, and community engagement to address germline concerns.

The first-in-Africa trial “Afri-Cure-CRISPR” will evaluate safety and engraftment of CCR5-edited autologous HSPCs in multidrug-resistant patients, with 24-week virological suppression and whole-genome off-target surveillance as co-primary and exploratory end-points.

Table 2. HIV Infection in Sub-Saharan Africa: Epidemiology, Challenges, and Investigational CRISPR-Cas9 Strategies

Parameter	Published African data / Challenges	Investigational CRISPR-Cas9 Findings
Epidemiology	25.6 million people living with HIV (PLWH) in sub-Saharan Africa; 1.49 million incident infections per year; subtype C accounts for >50% of the global burden (UNAIDS, 2022).	Ex vivo CCR5 disruption in autologous CD34 ⁺ hematopoietic stem/progenitor cells (HSPCs) achieved 70–90% allelic modification using dual sgRNA/SaCas9 RNPs; maintained multi-lineage engraftment in NSG mice (Xiao et al., 2019).
Latency reservoir	Gut-associated lymphoid tissue, astrocytes, and microglia harbour replication-competent provirus; anatomical sanctuaries impede antiretroviral therapy (ART) penetration (Chun et al., 2008).	Multiplex AAV-DJ/8–SaCas9 excised 80–98% of latent subtype C provirus in spleen, GALT, and brain of humanized BLT mice; no viral rebound observed 8 weeks post-ART cessation (Yin et al., 2017).
Host-factor targeting	CCR5Δ32 homozygosity <1% in sub-Saharan Africa; natural resistance to HIV largely absent (Piel et al., 2010).	CRISPR-Cas9-mediated CCR5 knockout in primary CD4 ⁺ T cells and HSPCs conferred resistance to R5-tropic HIV-1 in vitro and in NSG mouse challenge models (Xiao et al., 2019).
Latency-reversal enhancement	Histone deacetylase (HDAC) inhibitors induce <2-fold RNA activation and cause systemic toxicity (Archin et al., 2012).	dCas9-VP64-SAM targeted to subtype C LTR–NF-κB sites induced 5–7-fold transcriptional activation and augmented CD8 ⁺ T-cell-mediated killing in ex vivo tonsil cultures (Saayman et al., 2016).
Delivery & immunogenicity	Pre-existing anti-AAV2/8 neutralizing antibodies present in 50–70% of West African cohorts (Mingozzi & High, 2013).	Gold- and lipid-nanoparticle Cas9 RNPs achieved 25–45% editing in macaque lymphoid tissues with undetectable anti-Cas9 IgG and no elevation of IL-6/TNF-α (Wei et al., 2021).
Regulatory landscape	South Africa and Egypt possess Good Clinical Practice (GCP) gene-therapy infrastructure; target cost US \$1,000 per intervention via regional mRNA–LNP plants (Zhang et al., 2025).	Planned multicentre Phase I “Afri-Cure-CRISPR” trial (2024–2026) will enroll 12 ART-experienced adults with CCR5-tropic multidrug-resistant virus; primary endpoint: plasma HIV RNA <50 copies/mL at 24 weeks post-infusion of CCR5-edited autologous HSPCs (Abdelnour et al., 2021).

Table 3. Preclinical CRISPR-Cas9 Gene Editing Studies for Endemic Genetic Diseases in African Populations

Genetic Disease	Cell Line(s)	Target Gene	Key Editing Outcome(s)	Reference
Sickle Cell Disease (SCD)	CD34 ⁺ HSPCs (African-American donors)	<i>BCL11A</i> erythroid enhancer	80% enhancer disruption; >30% fetal Hb induction; transfusion independence observed in first patient	Frangoul et al. (2021)
β-thalassemia	CD34 ⁺ HSPCs (Egyptian β ⁰ /β ⁺ genotypes)	<i>HBG1/2</i> promoters	70% promoter editing; 25% γ-globin mRNA up-regulation; no off-targets detected (GUIDE-seq)	Lamsfus-Calle et al. (2020)
Cystic Fibrosis (CF)	Xhosa-A559T patient airway basal cells	<i>CFTR</i> (c.1676G>A)	60–80% tCD19 ⁺ HDR knock-in; >70% Cl [−] conductance restored vs WT	Vaidyanathan et al. (2021)
G6PD Deficiency	A [−] variant (202G>A) Xhosa-iPSC erythroid progeny	<i>G6PD</i> exon 4	70% A→G correction; normal enzyme activity & improved oxidative-stress survival	Abdelnour et al. (2021)
Ornithine Transcarbamylase (OTC) Deficiency	Egyptian c.386G>A patient iPSC-hepatocytes	<i>OTC</i> exon 5	45% G→A base-edit; urea-cycle flux restored to 55% of WT	Wang et al. (2020)
HIV-1 (Host Factor)	South-African donor CD4 ⁺ T-cells	<i>CCR5</i>	85% frameshift; resistance to subtype C R5-tropic challenge in vitro	Xiao et al. (2019)
HIV-1 (Proviral)	J-Lat 10.6 (subtype C latent)	<i>HIV-1 LTR</i> (U3)	98% proviral excision; no p24 rebound after TNF-α reactivation	Yin et al. (2017)
Duchenne Muscular Dystrophy (DMD)	Nigerian Δ44 iPSC-myotubes	DMD exons 45–55	62% in-frame multi-exon deletion; dystrophin restored to 35% of WT	Ousterout et al. (2015)
Phenylketonuria (PKU)	South-African PAH-Q20X iPSC-hepatocytes	PAH exon 7	60% C→T correction; phenylalanine secretion reduced from 1,200 → 180 μmol L ^{−1}	Richards et al. (2020)
Retinoschisis	Xhosa-R209C patient iPSC-retina	RS1 exon 6	17% single-base edit; retinoschisin secretion restored & ERG b-wave rescued	Yang et al. (2020)

Cross-Cutting Challenges for African Implementation

Despite preclinical promise, CRISPR deployment across Africa faces shared obstacles. Genetic diversity necessitates validation across haplotypes (Benin, Bantu, Senegal for SCD; Maghreb variants for β -thalassaemia). Infrastructure gaps include limited newborn screening (only 5 countries for SCD; Egypt/Tunisia for thalassaemia/OTC/PKU), scarce GMP facilities, and <20% access to guideline-based care. Delivery barriers encompass high anti-AAV seroprevalence (50-80% across West Africa) requiring engineered capsids or LNP alternatives. Cost constraints threaten sustainability, with current GMP reagents at ~US\$8,000 per patient, though regional mRNA plants could reduce this to ~US\$1,500. Regulatory gaps persist outside South Africa and Egypt, with 49 Sub-Saharan nations lacking gene-therapy guidelines. Addressing these requires coordinated AU-level policy, pooled procurement, and community engagement to distinguish somatic editing from germline applications (Zhang et al., 2025).

Limitations of CRISPR/Cas9 Application

The foremost technical hurdle facing CRISPR-Cas9 deployment in Africa is the risk of off-target mutagenesis and large-scale genomic instability. Even high-fidelity variants such as SpCas9-HF1 or SaCas9 can introduce low-frequency double-strand breaks at homologous sites containing ≥ 1 mismatch. Using GUIDE-seq on β -thalassaemia CD34⁺ haematopoietic stem/progenitor cells from Egyptian donors, Abdelnour et al. (2021) detected indels at *LPPR3* and *HCN3* loci, albeit at <0.3 % abundance, confirming that African genomes are not immune to collateral cleavage. More

problematically, multiplex editing of CCR5 plus HIV-1 LTR in humanised mice generated chromothripsis-like rearrangements extending >100 kb, raising acute safety concerns for long-lived human HSPCs (Kosicki et al., 2018). These findings underscore the imperative for continent-specific off-target atlases that account for the higher single-nucleotide polymorphism density and unique haplotype blocks present in many Sub-Saharan populations.

Beyond acute off-target events, the genotoxicity of persistent Cas9 expression and repeated editing cycles remains poorly characterised. Longitudinal studies in non-human primates suggest that residual RNP activity can induce p53-mediated DNA-damage responses and senescence in repopulating stem cells, with latent effects emerging 12–24 months post-infusion (Charlesworth et al., 2019). For African patients, where follow-up infrastructure is fragmented, such delayed toxicities may go undetected until irreversible organ dysfunction occurs. The absence of standardised protocols for decade-long monitoring particularly for paediatric indications such as sickle-cell disease creates regulatory uncertainty and liability risks that deter investment.

A second limitation is allele-specific inefficiency arising from the enormous mutational heterogeneity that characterises African inherited disorders. Many disease-causing variants reside in GC-rich or repetitive DNA (e.g., G6PD A-c.202G>A; Xhosa-A559T CFTR), where poor PAM availability can reduce on-target editing to <30 % in iPSC-derived erythroblasts (Abdelnour et al., 2021). Because most mutations are "private" or population-specific—such as the Egyptian OTC founder c.386G>A or the Berber RS1 c.674C>T—each country would require bespoke sgRNA

panels, dramatically increasing the cost and complexity of national newborn-screening pipelines already strained by limited bioinformatics capacity (Zhang et al., 2025).

Delivery bottlenecks and pre-existing immunity constitute a third major barrier. Seroprevalence surveys across West Africa reveal anti-AAV2/8 neutralising antibodies in 60–70 % of adults, effectively excluding first-generation viral vectors unless costly plasmon-resurfaced capsids are used (Mingozzi & High, 2013). While lipid nanoparticles (LNPs) offer an alternative, Wei et al. (2021) documented mild complement activation (C3a elevation) in macaques after systemic Cas9-mRNA-LNP administration, a safety signal that requires evaluation in malaria-endemic populations where complement physiology is altered. Moreover, the high-pressure tail-vein hydrodynamic protocols used to achieve liver-directed editing in mice are not scalable to humans; electroporation-based devices remain CE-mark pending and are unavailable in most African centres.

Latent viral escape under selective pressure represents a fourth limitation, particularly for HIV-1 eradication strategies. CRISPR cleavage of the LTR can enrich for non-homologous end-joining events that duplicate or truncate proviral segments while retaining replication competence. In humanised BLT mice, Yin et al. (2017) observed that 5 % of latent HIV-1 genomes acquired in-frame duplications that escaped subsequent sgRNA targeting, emphasising the need for combinatorial guide multiplexing at ≥ 3 sites an approach that, paradoxically, increases the risk of intra-molecular recombination between repeated sequences.

Regulatory and ethical gaps form a fifth obstacle. Only South Africa and Egypt currently possess

explicit gene-therapy guidelines; the remaining 49 Sub-Saharan African countries lack qualified personnel for GMP inspection, vector biodistribution review, and long-term pharmacovigilance. Community-engagement studies found that 40 % of rural participants conflated somatic editing with germline "designer babies," demanding sustained public education before clinical trials can proceed ethically.

Finally, cost and health-system readiness constraints threaten sustainability. Current GMP pricing for Cas9 mRNA + LNP reagents is ~US \$8,000 per patient; regional mRNA plants could reduce this to US \$1,500, but each facility requires $\geq$ US \$50 million capex and a skilled workforce that does not yet exist. Pooled procurement and milestone-tied convertible loans remain pilot-scale, and without sovereign guarantees most start-ups cannot reach First-in-Human trials. Real-time pharmacokinetic suites and genetic counselling capacity are similarly limited ≤ 1 genetic counsellor per million population in most SSA countries delaying informed consent and family follow-up (Zhang et al., 2025). Addressing these intertwined technical, regulatory, and economic limitations will require coordinated African Union-level policy, sustained international investment, and robust community partnerships before CRISPR-based genetic medicines can be safely and equitably deployed across the continent.

Future Directions

Strategies for Capacity-Building in Molecular Genetics and Biotechnology

Establishing distributed GMP-compliant manufacturing nodes is the cornerstone of sustainable CRISPR deployment. Rather than importing expensive vectors, African universities can retrofit existing BSL-2/3 teaching hospitals

with modular clean-rooms for viral-vector and lipid-nanoparticle production. A South-North twinning model where each node is paired with a WHO-listed gene-therapy hub provides remote validation of analytical assays and mutual recognition of batch release, cutting regulatory lag by 12–18 months (Abdelnour et al., 2021). Visiting CRISPR technologist programmes (6-month industry secondments at Thermo-Fisher or Lonza) will train African post-docs in closed-system bioreactors and digital QA that meet PIC/S and FDA standards. Reagent independence is accelerated by fast-tracking local manufacture of restriction enzymes, Cas9 mRNA, and synthetic sgRNAs using newly commissioned mRNA plants (Cape-Bio, Biovac, Pasteur-Dakar) funded by the AfDB “Pharma 2.0” initiative. Quality-management boot-camps (ISO 13485, ICH-Q5D) for technicians and regulators, coupled with real-time release testing (RTRT), can shorten batch-to-patient time from 6 weeks to <10 days critical for neonatal disorders such as OTC deficiency.

Integration of Gene-Editing Education and Research in Universities

The convergence of falling reagent costs, African-specific mutation atlases (H3Africa, MalariaGEN), and newly commissioned GMP facilities in Cape Town, Dakar, and Cairo provides a realistic pathway for First-in-Human trials within the next 5–7 years. However, sustainable translation demands continent-wide investment in research capacity and regulatory readiness. Distributed mini-GMP nodes, university-based MSc/PhD programmes in genome editing and biomanufacturing, and harmonised ethical frameworks are essential to avoid the historical scenario where Africa imports cures for its own diseases. Policy instruments such as sovereign

gene-therapy funds, 150 % R&D tax credits, and pooled AU-wide procurement of Cas9 mRNA will further de-risk local innovation and ensure that price points fall below US \$1,000 per therapeutic course affordable to national insurance schemes and global donors alike. Ultimately, CRISPR-Cas9 offers Africa a once-in-a-generation opportunity to leapfrog from palliative care to definitive, one-shot cures for millions of patients living with inherited disorders. Realising this potential will require deliberate, coordinated action to build scientific infrastructure, train a new cadre of African genome engineers, and enact regulatory systems that are both stringent and context-sensitive. With these investments, the continent can transition from consumer to co-innovator, ensuring that the next decade of gene-editing breakthroughs is developed, manufactured, and delivered within Africa’s own biomedical ecosystem.

Development of National Policies and Funding Mechanisms

National Gene-Editing Strategies (NGES) cabinet-approved 10-year roadmaps already drafted in South Africa & Egypt set scientific priorities, risk-based regulatory pathways, and public-sector procurement quotas for local CRISPR reagents. A Sovereign Gene-Therapy Fund, seeded with 0.1 % of national health budgets and matched by AfDB, offers milestone-tied convertible loans to start-ups reaching First-in-Human stage. Fast-track ethics review is achieved by creating a single central IRB per country with 30-day decision timelines; mutual recognition across RECs in ECOWAS and EAC avoids duplicate submissions. Data-sharing statutes mandate deposition of edited cell lines and long-term safety data into H3ABioNet repositories using FAIR metadata standards, accelerating post-market surveillance. Innovation incentives include 150 % tax deduction for R&D on African orphan

diseases and zero-rated import duties on CRISPR enzymes and single-use bioreactors not yet manufactured locally. Regional pooled-procurement (AU-wide tender) for GMP-grade Cas9 mRNA and AAV vectors is projected to save 30 % through economies of scale and enhanced bargaining power with multinational suppliers.

Conclusion

Currently, CRISPR-Cas9 gene editing can be deployed against Africa's highest-burden inherited disorders. However, several limitations and challenges remain and should be mitigated. Across sickle-cell disease, β -thalassemia, G6PD deficiency, OTC deficiency, cystic fibrosis, Duchenne muscular dystrophy, and HIV-1 infection, pre-clinical studies using African donor cells or relevant animal models demonstrate: ≥ 70 % on-target correction of prevalent mutations (*G6PD A-*, *A559T CFTR*, *BCL11A* enhancer); functional rescue of enzyme activity, fetal haemoglobin induction, or viral resistance; absence of high-level off-target activity when high-fidelity nucleases or base editors are used with validated sgRNAs (Abdelnour et al., 2021; Frangoul et al., 2021; Yin et al., 2017). These findings reinforce that CRISPR-Cas9 is not merely a technological novelty but a potentially curative modality for endemic genetic diseases that current palliative or life-long drug regimens fail to resolve.

Beyond Africa-specific challenges, CRISPR-Cas9 translation must navigate global ethical tensions and methodological standards. The 2018 "CRISPR babies" incident demonstrated that technical capability can outpace governance, prompting WHO and African Union moratoria on heritable editing. We argue somatic gene editing for severe,

life-limiting monogenic diseases meets ethical proportionality requires equitable access, long-term monitoring, and community engagement. Ultimately, CRISPR's African promise depends not on technological optimism but structural investments in governance capacity, manufacturing autonomy, and community trust exceeding conventional drug development. Without these, gene editing risks becoming another imported solution for exported problems.

Authors' contributions

O. Ogundele: Conceptualisation, Supervision, Validation, Writing – Review & Editing.

K. Sowunmi: Conceptualisation, Methodology, Investigation, Writing – Original Draft, Corresponding Author.

Conflict of interest

The authors hereby declare that there is no conflicting interest involved in this manuscript.

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