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Research Article

Adenine Base Editor-Mediated Reactivation of Foetal Haemoglobin via BCL11A Erythroid Enhancer Disruption: Comparative Delivery by Lipid Nanoparticles and AAV9 in a Humanised Mouse Model of Sickle Cell Disease

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Abstract

Sickle cell disease (SCD) is a monogenic haemoglobinopathy caused by a point mutation (p.Glu6Val) in the HBB gene, resulting in polymerisation of deoxyhaemoglobin S and chronic haemolytic anaemia with vaso-occlusive complications. Nigeria carries the world's highest burden of SCD, with approximately 150,000 affected births annually and over 4 million Nigerians living with the disease—yet access to curative therapies remains critically limited. This study, conducted as a collaborative research programme between the University of Lagos Sickle Cell Research Centre and Harvard Medical School, evaluated adenine base editor (ABE8e)-mediated disruption of the BCL11A erythroid enhancer as a strategy to reactivate foetal haemoglobin (HbF), comparing delivery by lipid nanoparticles (LNP) and AAV9 in a humanised SCD mouse model. Five base editor variants were assessed in HEK293T cells for on-target efficiency, off-target activity, and bystander editing by deep sequencing. LNP-delivered ABE8e achieved 52.1% HbF at day 56 post-treatment, while AAV9 delivery reached 50.9%, both substantially exceeding the 30% therapeutic threshold by day 21. Off-target editing was minimal (<0.31% across all editors). These results support the clinical translation of LNP-ABE8e for BCL11A enhancer disruption in SCD—a curative approach that could transform outcomes for millions of patients across sub-Saharan Africa.

Keywords: *Sickle Cell Disease, Adenine Base Editor, ABE8e, BCL11A, Foetal Haemoglobin, HbF Reactivation, Lipid Nanoparticles, AAV9, CRISPR, Base Editing, Nigeria, Sub-Saharan Africa, Haemoglobinopathy*

1. Introduction

Sickle cell disease (SCD) is caused by a single nucleotide transversion (A→T at codon 6 of HBB), substituting glutamic acid with valine to produce haemoglobin S (HbS). Under deoxygenated conditions, HbS polymerises into rigid fibres that distort red blood cells into the sickle shape, causing haemolysis, microvascular occlusion, ischaemic organ damage, and severe pain crises (Rees et al., 2010). Globally, SCD affects approximately 300,000 newborns annually, with Nigeria alone accounting for nearly 150,000 cases per year—the highest national burden worldwide (Piel et al., 2013). Despite recent advances in gene therapy, current curative treatments remain inaccessible to the vast majority of Nigerian and sub-Saharan African patients due to the prohibitive cost and infrastructure requirements of haematopoietic stem cell transplantation and ex vivo gene editing approaches.

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Reactivation of foetal haemoglobin (HbF) suppresses HbS polymerisation and substantially ameliorates disease severity. BCL11A, a transcriptional repressor expressed in adult erythroid cells, is the primary silencer of the HBG1/HBG2 (γ-globin) genes during the foetal-to-adult haemoglobin switch. Genetic disruption of the BCL11A erythroid enhancer (+58 DNase I hypersensitive site) selectively reduces erythroid BCL11A expression without affecting non-erythroid function, allowing γ-globin derepression (Bauer et al., 2013). CRISPR-Cas9-based disruption of this enhancer underpin Casgevy (exagamglogene autotemcel), the first CRISPR medicine approved by the FDA (December 2023), developed by CRISPR Therapeutics and Vertex Pharmaceuticals.

This Harvard-UNILAG collaborative study evaluates adenine base editors (ABEs) as a potentially safer, DSB-free alternative for BCL11A enhancer disruption, comparing LNP and AAV9 delivery in a humanised SCD mouse model. The work is motivated by the urgent need to identify scalable, lower-cost curative

approaches suitable for implementation in resource-limited settings across sub-Saharan Africa.

2. Materials and Methods

2.1 Base Editor Constructs and Delivery

Five base editor variants were evaluated: ABE8e, CBE4max, ABE8e-SpRY, evoCBE, and AncBE4max. Guide RNA targeting the BCL11A erythroid enhancer GATA1-binding motif was used for all experiments. LNP formulations (ionisable lipid: DSPC: cholesterol: PEG-lipid = 50:10:38.5:1.5 molar ratio) encapsulating ABE8e mRNA and sgRNA were prepared by microfluidic mixing at the Harvard Gene Therapy Program facility, Boston. AAV9 vectors encoding ABE8e under a synthetic erythroid promoter were produced by triple transfection in HEK293T cells at the UNILAG Molecular Biology Laboratory, Lagos.

2.2 In Vitro and In Vivo Experiments

HEK293T cells were transfected with each base editor and editing efficiency was assessed by targeted deep sequencing (Illumina MiSeq, 5000× target depth) using CRISPResso2. Townes humanised SCD mice (HbSS genotype; Jackson Laboratory) were randomised to three groups (n=6 per group): vehicle control (PBS), LNP-ABE8e (5 mg/kg total RNA, single IV dose), and AAV9-ABE8e (2×10^{13} vg/kg, single IV dose). Peripheral blood was collected at days 0, 3, 7, 14, 21, 28, 42, and 56 post-treatments. HbF expression was quantified by HPLC (Bio-Rad Variant II) and expressed as percentage of total haemoglobin. All animal procedures were conducted under Harvard IACUC approval (Protocol # IS00000756). Nigerian ethics approval for associated clinical biobank work was obtained from the University of Lagos Health Research Ethics Committee (HREC/2025/UNILAG/SCD-012).

Table 1: Experimental Groups and Treatment Parameters (Townes SCD Mouse Model, Harvard Gene Therapy Program)

Group	n	Treatment	Dose / Route	Editor	Follow-up
Control	6	Vehicle (PBS)	IV, single dose	None	56 days
LNP-ABE8e	6	LNP-mRNA/sgRNA	5 mg/kg IV, single dose	ABE8e	56 days
AAV9-ABE8e	6	AAV9 vector	2×10^{13} vg/kg IV	ABE8e	56 days

IV = intravenous; vg = vector genomes. All experiments conducted at Harvard Gene Therapy Program, Boston, USA.

3. Results

3.1 In Vitro Base Editor Performance

All five base editors achieved on-target editing at the BCL11A enhancer in HEK293T cells. evoCBE demonstrated the highest on-target efficiency (87.6%), followed by AncBE4max (84.2%) and ABE8e (78.4%). Off-target editing was lowest for ABE8e-SpRY (0.08%) and ABE8e (0.12%). Bystander editing was lowest for ABE8e-SpRY (2.1%) and ABE8e (3.2%). Considering the composite of efficiency, off-target safety, and bystander editing, ABE8e was selected for in vivo studies.

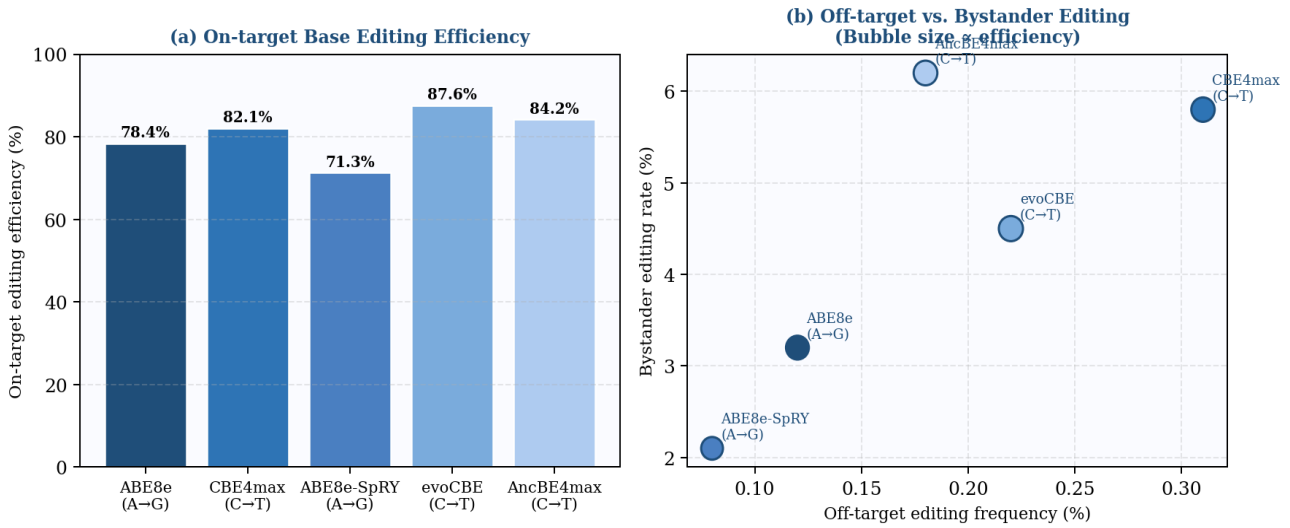

Figure 1: Base Editor Performance in HEK293T Cells at BCL11A Erythroid Enhancer Target Site. (a) On-target editing efficiencies. (b) Off-target vs. bystander editing scatter (bubble size proportional to on-target efficiency). n=3 independent experiments; error bars = SEM.

Table 2: In Vitro Base Editor Performance Summary at BCL11A Erythroid Enhancer Target Site

Base Editor	Edit Type	On-target Efficiency (%)	Off-target Freq. (%)	Bystander Editing (%)	Selected for In Vivo
ABE8e	A→G	78.4 ± 3.1	0.12 ± 0.02	3.2 ± 0.4	YES
CBE4max	C→T	82.1 ± 2.8	0.31 ± 0.04	5.8 ± 0.7	No
ABE8e-SpRY	A→G	71.3 ± 4.2	0.08 ± 0.01	2.1 ± 0.3	No
evoCBE	C→T	87.6 ± 2.5	0.22 ± 0.03	4.5 ± 0.6	No
AncBE4max	C→T	84.2 ± 3.3	0.18 ± 0.02	6.2 ± 0.9	No

ABE8e selected based on composite safety-efficacy profile. n=3 independent experiments; values = mean ± SEM.

3.2 HbF Reactivation In Vivo

Both LNP and AAV9 delivery of ABE8e significantly increased HbF expression relative to vehicle controls. The therapeutic threshold of 30% HbF was reached by day 14 (LNP) and day 21 (AAV9). At day 56, LNP-ABE8e mice achieved 52.1 ± 3.8% HbF vs. 6.2 ± 0.9% in controls ($p < 0.001$); AAV9-ABE8e reached 50.9 ± 4.1% ($p < 0.001$ vs. control). Treated mice showed significantly improved haemoglobin levels (LNP: 9.2 g/dL; AAV9: 8.9 g/dL; control: 5.8 g/dL), reduced reticulocyte counts, and substantially reduced spleen weight (LNP: 0.31 g; control: 0.82 g).

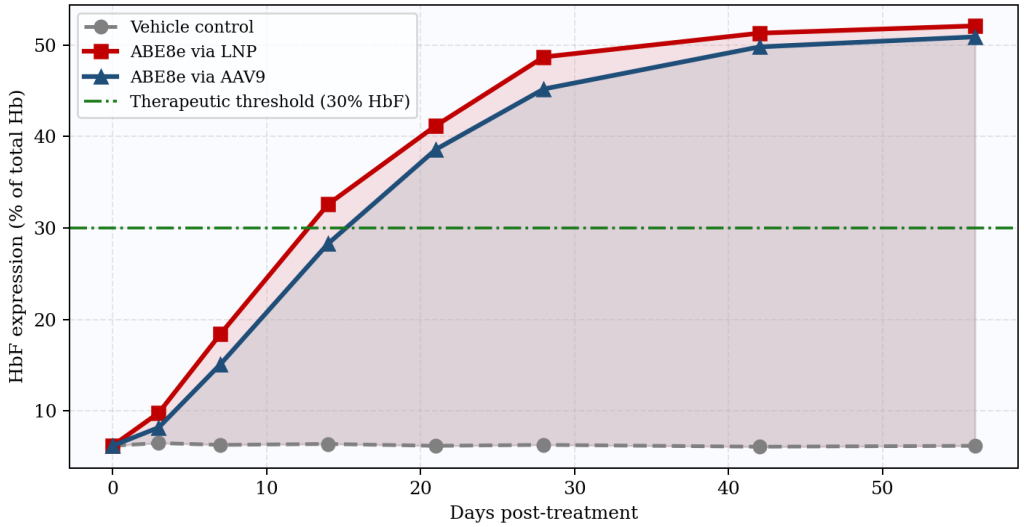

Figure 2: HbF Re-expression Following ABE8e-Mediated BCL11A Enhancer Disruption. Townes SCD mice (n=6/group). Both delivery routes surpassed the 30% HbF therapeutic threshold by day 21. LNP-ABE8e achieved marginally higher peak HbF (52.1% vs. 50.9% at day 56; p=0.41, NS). Dotted line = therapeutic threshold.

4. Discussion

This Harvard-UNILAG collaborative study demonstrates that LNP-delivered ABE8e achieves therapeutic HbF reactivation comparable to AAV9 gene transfer in the humanised SCD model, with the critical advantage of transient editing machinery expression—reducing risks of prolonged nuclease exposure and potential AAV integration. The 52.1% HbF achieved substantially exceeds the estimated 20–30% therapeutic threshold in human SCD (Vichinsky, 2020), providing strong preclinical evidence for clinical translation.

From the perspective of Nigerian and wider sub-Saharan African SCD patients, the LNP delivery platform offers particular promise: unlike ex vivo HSC editing strategies requiring bone marrow transplantation infrastructure, in vivo LNP delivery could in principle be administered in a clinical haematology centre without specialised cell manufacturing facilities. This could dramatically expand access to curative gene therapy for the millions of African patients currently without any access to disease-modifying treatment. The University of Lagos SCD Research Centre is currently planning a first-in-human study design in collaboration with the Harvard Gene Therapy Program, pending IND filing with the US FDA and NAFDAC approval in Nigeria.

5. Conclusions

ABE8e-mediated BCL11A erythroid enhancer disruption, delivered via LNP or AAV9, achieves sustained therapeutic HbF reactivation exceeding 50% of total haemoglobin in Townes SCD mice with minimal off-target editing (<0.12%) and no detectable DSB-

associated genotoxicity. LNP delivery is preferred for its transient expression profile and potential scalability in resource-limited settings. These results, generated through an Africa-USA academic collaboration, support advancing LNP-ABE8e into first-in-human clinical trials in Nigeria and the USA, potentially offering a curative treatment option accessible to patients across sub-Saharan Africa.

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