Dear Editor, Replacing or restoring function in mutant genes in the skin of patients with genodermatoses represents a major focus in translational research. For patients with the inherited blistering disease, recessive dystrophic epidermolysis bullosa (RDEB), such endeavours have advanced with the development and partial licensing of a topical $COL7A1$ gene replacement therapy, which transiently restores the missing type VII collagen (C7) to RDEB wounds and promotes wound healing. However, in search of permanent gene correction in RDEB, we have pursued a gene repair approach using $COL7A1$ gene editing, focusing on the development and application of base editing.

Base editing avoids the risk of double-stranded DNA breaks that is associated with CRISPR-Cas9 and other homology-directed repair approaches. Utilizing a nickase Cas9 fused to either a cytidine or adenine deaminase, base editing can catalyse base transitions at specific sites determined by a single guide RNA (sgRNA). This base transition triggers mismatch repair complexes to correct the mismatch on each strand, resulting in full base-pair conversion. Adenine base editors (ABEs) catalyse the transition from adenine to guanine, and ABE8e is a novel form of ABE that, through phage-assisted evolution, has higher deamination kinetics than other forms of ABE, and thus higher editing efficiency. We have previously reported high efficiency in editing pathogenic variants in $COL7A1$ in RDEB fibroblasts with minimal off-targets effects using ABE8e.

Our goal is to develop topical base editing creams or ointments for RDEB and other genodermatoses. Here, in the next phase of our preclinical work, we explored the use of lipid delivery systems (in contrast to earlier electroporation delivery) to effect base editing in RDEB fibroblasts. We used primary fibroblasts from two patients with RDEB harbouring the homozygous $COL7A1$ variant, c.4448G>A (p.Gly1483Asp) (Figure 1a). This missense change in $COL7A1$ has previously been shown to result in intermediate or localized RDEB (when homozygous), but also, in some heterozygous individuals, it can result in a self-improving or localized form of dominant DEB. Skin biopsy from our patients showed reduced baseline C7 expression at the dermoepidermal junction (Figure 1b), although Western blotting showed no overall reduction in C7 levels compared with control fibroblasts.

For $COL7A1$ gene editing, we used 5 µL lipofectamine MessengerMax™ (Thermo Fisher Scientific Inc., Waltham, MA, USA) in 250 µL Opti-MEM to deliver small doses of ABE8e mRNA and sgRNA into 200 000 primary fibroblasts using reverse transfection. Two different guides were designed with BE-designer to target the $COL7A1$ locus for base editing, both of which were equally effective (Figure 1c). We selected sgRNA-1 to deliver a range of doses of ABE8e (50–400 ng per 200 000 cells) to patient fibroblasts. The ratio of sgRNA-1 to ABE8e mRNA was 1 : 7 based on previous optimization experiments. We identified 400 ng ABE8e as the dose needed for 80–90% editing efficiency of the $COL7A1$ variant, as demonstrated in both patients (Figure 1d–f), whereas ~40–60% editing efficiency could be achieved using 100 ng ABE8e. This dosage-dependent editing efficiency was further corroborated via next-generation sequencing (NGS) of the on-target site using the Illumina MiSeq platform (Illumina, San Diego, CA, USA) (Figure 1g), analysed as previously reported. Contrary to other studies, no bystander mutations were observed around the site of editing, in either Sanger sequencing or NGS, due to the absence of another A base within the editing window.
Figure 1 Base editing corrects COL7A1 c.4448 G > A homozygous variants with high efficiency. (a) Sanger sequencing confirms homozygous c.4448 G > A mutation in two patients. (b) Blistering is observed in a biopsy of a patient with epidermolysis bullosa (EB) due to reduced C7 at the dermoepidermal junction. Scale bars = 100 µm. (c) Two guides were designed for base editing to be used with synthesized ABE8e mRNA to correct the COL7A1 c.4448 G > A variant. They show comparable editing efficiencies. (d) ABE8e confers reliable gene editing as it comparably and efficiently edits two independent patients’ cells. (e, f) By varying doses of ABE8e, we achieved increased editing efficiency as detected by sanger sequencing. (g) Next-generation sequencing analysis illustrates the impact of varying the ABE8e dose, with the average editing efficiency increasing from 25% to 90% when using 50–400 ng of the base editor. n = 1 for NHF (normal human fibroblasts), patient and 50 ng of ABE8e; n = 3 for 100 ng, 200 ng and 400 ng of ABE8e. *P = 0.027 (left), **P = 0.024 (right). (h) Despite there being no significant difference in C7 production, Western blot analysis of cell supernatant shows increased C7 secretion after editing (P = 0.059; close to significance); n = 3. Loading control: β-actin. (i) Three-dimensional skin equivalents (SE) using normal human keratinocytes (KC) and fibroblasts (FB) were used to deliver green fluorescent protein (GFP) mRNA using lipofectamine. The white arrows indicate GFP-positive cells. DAPI, 4',6-diamidino-2-phenylindole; E, epidermal layer; D, dermal layer. Scale bars = 100 µm.
Three Western blots of patient fibroblast lysate and supernatant demonstrated an increase in C7 production and secretion in edited patient fibroblasts despite no significant changes from baseline (Figure 1h). As the goal is to bring this methodology to an in vivo study, we have topically treated 1 cm² of three-dimensional skin equivalent (SE), constructed with normal human keratinocytes and fibroblasts, with 0.3 μg mRNA-based green fluorescent protein (GFP), using 0.5 μL lipofectamine in 34.2 μL Opti-MEM medium, applied dropwise to the SE. GFP-positive cells were visualized with direct fluorescence 24 h after the mRNA GFP was topically delivered onto SEs; efficacy was estimated at around 5% (Figure 1i). The SEs were constructed and harvested for analysis as previously reported.

In this study, we achieved 90% editing efficiency for biallelic correction of a homozygous variant in COL7A1 using ABE8e mRNA and lipofectamine as a delivery method in vitro. We also demonstrated the potential use of lipid nanoparticles (LNPs) like lipofectamine for in vivo topical therapies. Previous mRNA-LNP research has highlighted the need for care in optimizing LNPs to target and deliver mRNA cargos for efficacy and immunogenicity. Therefore, other lipid agents with fewer toxic side-effects should be tested for potential topical deliveries, ideally with peptide modifications that grant specificity for patient dermal or epidermal cells.

In conclusion, by achieving high efficiency in correcting COL7A1 c.4448 G > A, our work underscores the translational potential for developing this route to novel in vivo therapies for DEB and other skin diseases.

References