Base-Editing as a Therapeutic Approach for the Direct Correction of Disease-Causing Mutations underlying Glycogen Storage Disease Type Ia

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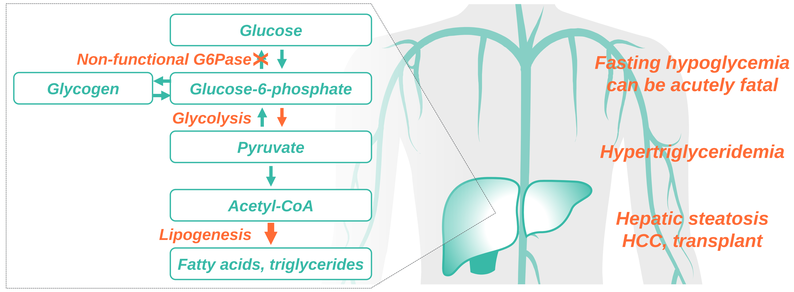
Disease Manifestations of Glycogen Storage Disease Type IA

Glycogen Storage Disease Type Ia (GSDIa) is an autosomal recessive disorder caused by mutations in the *G6PC* gene that disrupt a key enzyme, G6Pase, in glucose homeostasis[1]. GSDIa patients have fasting hypoglycemia, and accumulation of glycogen and fat in the liver, and in the kidneys, resulting in hepatomegaly and nephromegaly. There is no cure. Acutely fatal hypoglycemia is prevented by dietary supplementation every 1-4 hours with meticulous adherence, but the long-term complications of renal disease and hepatocellular carcinoma are not addressed. The two most prevalent *G6PC* mutations associated with GSDIa are R83C and Q347X, both containing a single G>A transition mutation. Adenine base editors (ABEs) enable the programmable conversion of A•T to G•C in genomic DNA and in principle could be used to precisely correct these mutations. Here, we engineered novel adenine base editors (ABE) variants for validation in pre-clinical models of GSD1a.



Genome Editing with Adenine Base Editors

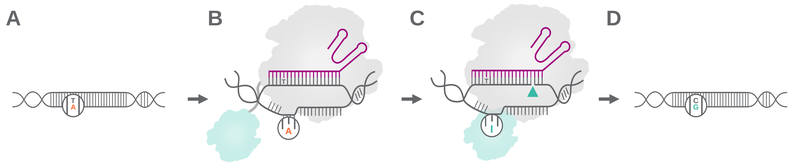


Figure 1. (A) Starting DNA sequence with the target base pair (A:T). (B) The adenine base editor (ABE) is a fusion protein consisting of an evolved TadA* deaminase (teal) connected to CRISPR-Cas enzyme (grey)[2,3]. The base editor binds to a target sequence that is complementary to the guide-RNA (magenta) and exposes a stretch of single-stranded DNA. (C) The deaminase converts the target adenine into inosine (which is read as guanine by DNA polymerases) and the Cas enzyme nicks (▲) the opposite strand. (D) The nicked strand is repaired completing the conversion of an A:T to G:C base pair.

Optimizing an ABE and gRNA to Correct the G6PC-R83C Mutation

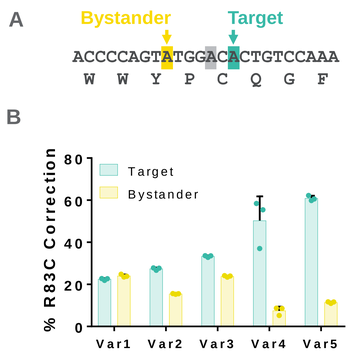


Figure 2. (A) Target DNA sequence and underlying amino acid translation for the GSD1a R83C mutation. Targeted edit is highlighted in teal, bystander in yellow, and an edit that could result in a synonymous conversion in gray. (B) Allele frequencies assessed by high-throughput targeted amplicon Next-Generation Sequencing (NGS) in HEK293T cells, harboring the G6PC transgene with the R83C mutation via lentiviral transduction, post transfection with base-editor mRNA and gRNA. Variants (Var) 1 – 5 represent a subset of the combinations of gRNA and base-editor mRNA, engineered for optimizing targeted correction and limit bystander conversion.

Optimizing an ABE and gRNA to Correct the G6PC-Q347X Mutation

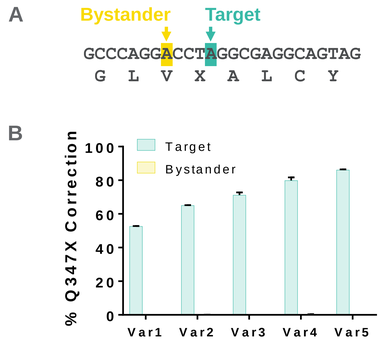


Figure 3. (A) Target DNA sequence and underlying amino acid translation for the GSD1a Q347X mutation. (B) Allele frequencies assessed by NGS in HEK293T cells, harboring the G6PC transgene with the Q347X mutation via lentiviral transduction, post transfection with base-editor mRNA and gRNA. Variants (Var) 1 – 5 represent a subset of combinations of gRNA and base-editor mRNA, engineered for optimizing targeted correction. Variants yield significant targeted correction; bystander editing is not detectable via NGS.

Precise Correction of GSD1a Mutations in Primary Human Hepatocytes

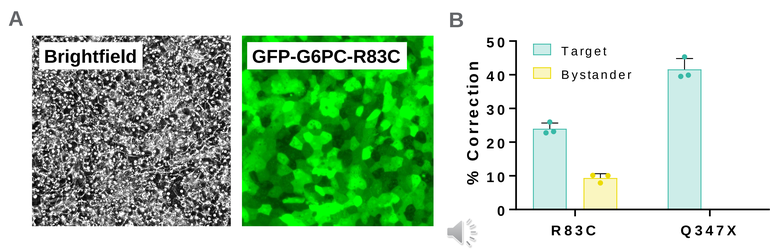


Figure 4. A long-lived primary human hepatocyte system was generated[4], harboring the G6PC transgene with either the R83C or Q347X mutation via transduction using a GFP-expressing lentiviral vector. (A) Representative 10x brightfield and fluorescence images shown for a hepatocyte monolayer. Post transduction, hepatocytes underwent transient transfection with base-editor mRNA and gRNA, using variant 5 systems screened in HEK293T cells, as noted in figures 2 and 3. (B) Allele frequencies assessed by NGS reveal significant target editing, resulting in the correction of R83C and Q347X mutations, at ~25% and ~40%, respectively, in the physiologically-relevant hepatocyte context.

Clinically-Relevant Levels of Correction of GSD1a Mutations in Patient iPS-derived Hepatocyte Like Cells

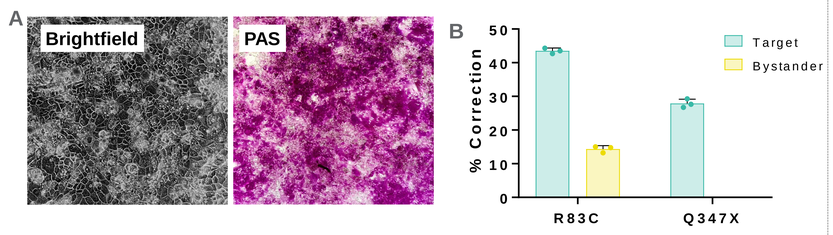


Figure 5. Patient iPS-derived hepatocyte like cells, either homozygous for R83C or compound heterozygous for Q347X (DefiniGen), were transiently transfected with base-editor mRNA and gRNA, using lead Variant 5 systems as described earlier. Base-editing outcomes. (A) Representative 4x brightfield and Periodic Acid-Schiff (PAS) stained cell monolayers reveal glycogen accumulation in cells sourced from the homozygous R83C patient. (B) Allele frequencies assessed by NGS reveal significant target editing, resulting in the correction of R83C and Q347X mutations, respectively.

In Vivo Correction of GSD1a Mutations in Livers of Transgenic Mouse Models, Heterozygous for either huG6PC-R83C or huG6PC-Q347X

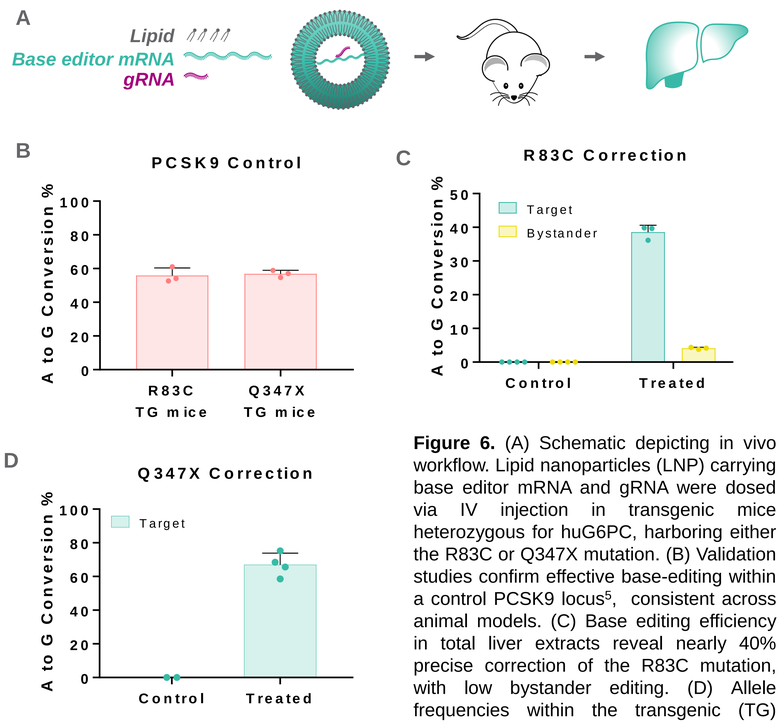


Figure 6. (A) Schematic depicting in vivo workflow. Lipid nanoparticles (LNP) carrying base editor mRNA and gRNA were dosed via IV injection in transgenic mice heterozygous for huG6PC, harboring either the R83C or Q347X mutation. (B) Validation studies confirm effective base-editing within a control PCSK9 locus[5], consistent across animal models. (C) Base editing efficiency in total liver extracts reveal nearly 40% precise correction of the R83C mutation, with low bystander editing. (D) Allele frequencies within the transgenic (TG) mice, heterozygous for huG6PC-Q347X, reveal ~70% correction. Bystander editing is not-detectable via NGS.

Conclusions & Future Directions

We engineered and validated novel adenine base editors (ABE) that achieve high level of precise correction of the two most prevalent GSD1a mutations, R83C and Q347X, in both in vitro and in vivo settings. Our data from transgenic mouse models reveal significant levels of mutation correction that surpass those expected to restore glucose homeostasis[1, 6-8]. This extent of correction for GSD1a mutations is greater than levels achieved by HDR-based methodologies, and editing is achieved without introduction of double stranded breaks. This demonstrates that our base-editing technology is an effective approach for the precise correction of mutations underlying GSDIa and highlight its therapeutic potential. We have initiated functional studies to correlate pathophysiology to extent of mutation correction by base-editing. Off-target analysis for the described guide RNA and base-editors has been initiated.

References and Disclosures

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Yvonne Aratyn-Schaus, Thomas Fernandez, Michael Packer, Genesis Lung, Lo-I Cheng, Sarah Smith, Krishna Ramanan, J. Robert Dorkin, Delai Chen, Giuseppe Ciaramella, and Francine M Gregoire are all employees of Beam Therapeutics.