



## Base editing: a brief review and a practical example

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### Abstract

Genome editing has undergone rapid development in recent years, yielding new approaches to make precise changes in genes. In this review, we discuss the development of various adenine and cytosine base-editing technologies, which share the ability to make specific base changes at specific sites in the genome. We also describe multiple applications of base editing *in vitro* and *in vivo*. Finally, as a practical example, we demonstrate the use of a cytosine base editor and an adenine base editor in human cells to introduce and then correct a prevalent mutation responsible for hereditary tyrosinemia type 1.

**Keywords:** base editing, CRISPR, genetics, genome editing, mutation

### Introduction

Precision genome editing is a growing field with industrial, agricultural, and biomedical applications. One of the dominant genome-editing systems available today is clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein 9 (Cas9). Through the use of a guide RNA (gRNA) with a sequence homologous to that of a sequence of DNA in the target genome (known as the protospacer) adjacent to a specific protospacer-adjacent motif (PAM) in the DNA, Cas9 can be used to create a double-strand break (DSB) at the targeted sequence<sup>[1]</sup>. Non-homologous end joining at DSBs can be used to create indels and knock out genes at genetic loci; likewise, homology-directed repair (HDR) can be used, with an introduced template DNA, to insert genes or modify the targeted sequence<sup>[2]</sup>. A variety of Cas9-based tools have been developed in recent years,

including tools that methylate DNA, recognize the broader sequence space, or create single-strand nicks. In this review, we describe Cas9-based tools that can generate specific point mutations in DNA without needing to create DSBs: base editors. We describe published examples of their use *in vitro* and *in vivo*, and we then present a specific application of such tools.

### Cytosine base editing

In 2016, Komor *et al* described the use of CRISPR-Cas9 to convert a cytosine base to a thymine base without introducing a template DNA strand<sup>[3]</sup>. After the cytidine deaminase domain of rat APOBEC1 was fused to the N-terminus of catalytically-dead Cas9 (dCas9) using the linker XTEN (resulting in a fusion protein called base editor 1, or BE1), conversion of cytosine to uracil was observed between position 4

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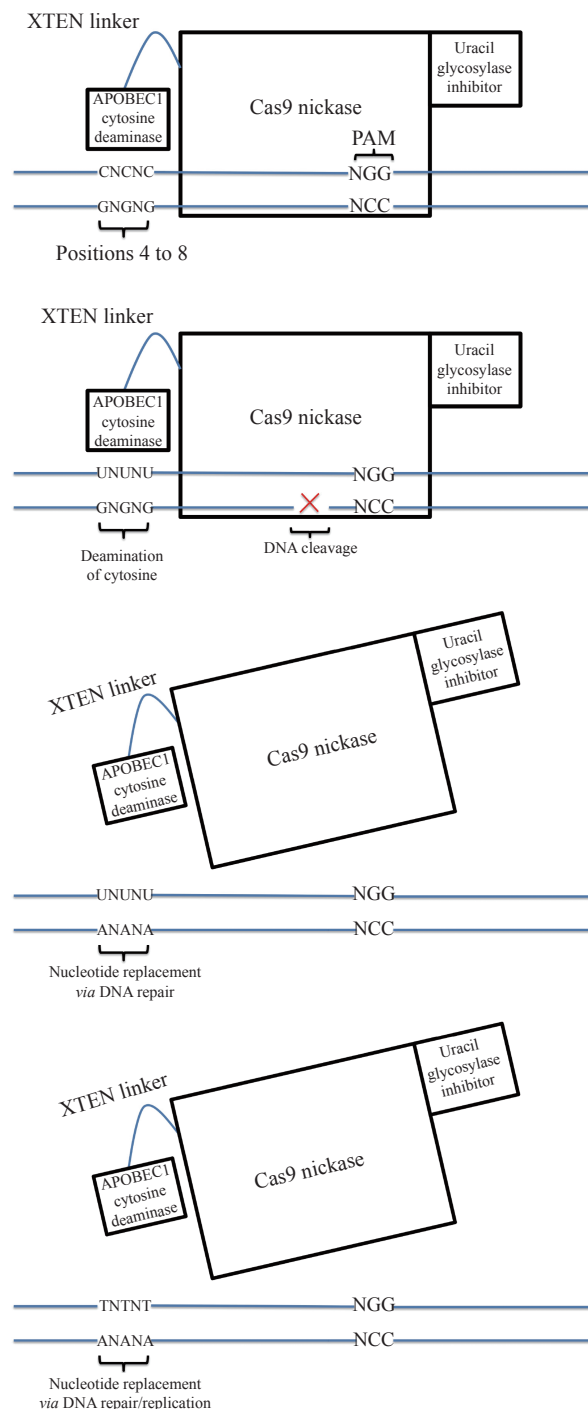
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and position 8 within the 20-nt protospacer region of DNA (or, to express it a different way, 13<sup>th</sup> to 17<sup>th</sup> nucleotides upstream of the PAM). Of note, any cytosine base within this "window" was amenable to editing, resulting in varied outcomes depending on which cytosines were edited and how many they were. After DNA replication or repair, each uracil was replaced by thymine, completing the C→T base editing<sup>[3]</sup>. The next version of base editor (BE2) incorporated a uracil glycosylase inhibitor fused to the C-terminus of dCas9 to help inhibit base excision repair of the uracil bases resulting from the cytidine deaminase activity (which otherwise would act to restore the original cytosine bases); this improved the efficiency of C→T base editing. The final version, BE3, used a Cas9 nickase rather than dCas9; the nickase cut the unedited strand opposite the edited C→T bases, stimulating the removal of the opposing guanidine through eukaryotic mismatch repair (**Fig. 1**). BE2 and BE3 base editing was observed in both human and murine cell lines. The specificity of base editing has been further improved through the addition of mutations to the Cas9 nickase<sup>[4]</sup>; in a similar fashion, Cas9 has been mutated to narrow the width of the editing window from approximately 5 nucleotides to as little as 1–2 nucleotides<sup>[5]</sup>.

An alternative cytosine base editing platform was described by Nishida *et al* in the same year<sup>[6]</sup>. By linking the activation-induced cytosine deaminase (AID) domain PmCDA1 to dCas9 (Target-AID), they were able to demonstrate targeted C→T base editing in yeast. Furthermore, an alternative C→T editing strategy was also demonstrated without fusing a deaminase domain to Cas9; instead, a SH3 (Src homology 3) domain was added to the C-terminus of dCas9 while a SHL (SH3 interaction ligand) was added to PmCDA1<sup>[6]</sup>. Optimization of efficiency was achieved through the use of a Cas9 nickase rather than dCas9, similar to the strategy employed by Komor *et al*<sup>[3]</sup>. Further, a uracil DNA glycosylase inhibitor was added to enhance base editing in the mammalian CHO cell line. The resulting platform was able to consistently edit bases within 3 to 5 bases of the 18<sup>th</sup> nucleotide upstream of the PAM sequence.

A distinct cytosine base editing platform was reported by Li *et al* in 2018<sup>[7]</sup>. Unlike the former two editors, Li *et al* used Cpf1 (also known as Cas12a) instead of Cas9 as the RNA-guided endonuclease. Catalytically-inactive Cpf1 was fused to APOBEC1 (dLbCpf1-BE0), leading to C→T conversion in a human cell line. While the Cas9 base editor variants BE3 and Target-AID recognize the PAM sequence NGG, dLbCpf1-BE0 recognizes the T-rich PAM



**Fig. 1 Schematic of mechanism of action of BE3, a cytosine base editor.** A: adenine; C: cytosine; G: guanine; T: thymine; U: uracil; N: any base

sequence TTTV. Although base editing was observed between positions 8 and 13 of the protospacer sequence with dLbCpf1-BE0, the introduction of additional mutations into Cpf1 was able to reduce the window to positions 10 to 12<sup>[7]</sup>. However, narrowing of the base editing window correlated with a decrease in editing efficiency.

Cytosine base editing is not wholly predictable;

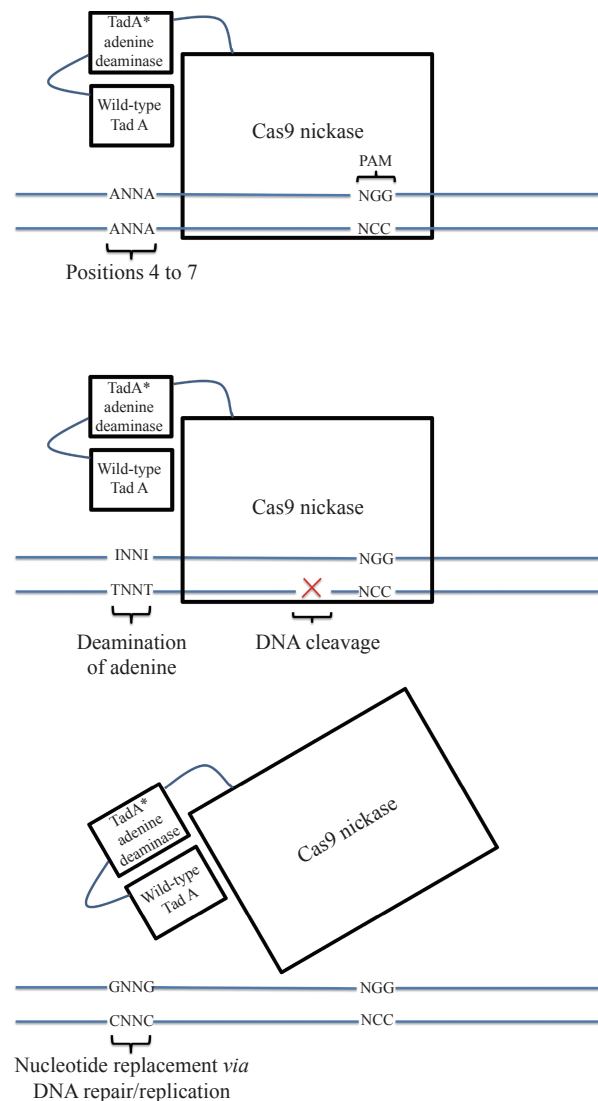
indels can occur at the target site, albeit at lower frequencies than those observed for C→T editing editors<sup>[3,6]</sup>. Furthermore, cytosine base editors can occasionally cause C→A or C→G edits rather than the expected C→T edits. To address this problem, Komor *et al* made three changes to the standard BE3 base editor<sup>[8]</sup>. The linker between Cas9 nickase and the rat APOBEC1 cytosine deaminase domain was increased from 16 amino acids to 32 amino acids, the linker between the Cas9 nickase and the uracil glycosylase inhibitor was increased from 4 amino acids to 9 amino acids, and a second uracil glycosylase inhibitor was appended to the C-terminus of the new cytosine base editor using another 9 amino acid linkers. This improved cytosine base editor was termed "BE4", in keeping with the BE(x) naming convention. Upon comparison to BE3 in HEK 293T cells across multiple loci, BE4 showed a 1.5-fold increase in C→T editing over BE3, whereas C→A and C→G edits decreased by 2.3 folds; indels were also reduced by 2.3 folds. A variant using Cas9 nickase derived from *Staphylococcus aureus* (rather than *Streptococcus pyogenes*, which is the most commonly used version) was also created, showing an increase in editing efficiency by 1.4 folds and a decrease in non-C→T edits by 1.8 folds over the standard BE3<sup>[8]</sup>. This variant was named SaBE4. Furthermore, in light of the hypothesis that Gam protein could protect against indels by binding to the ends of DSBs, Gam was fused to the N-termini of BE4, SaBE4, BE3, and SaBE3. Frequencies of indels and non-C→T edits for the Gam fusion proteins were universally lower than those for their non-Gam counterparts<sup>[8]</sup>.

## Adenine base editing

Cytosine base editors permit the introduction of C→T changes in the coding sequences of genes; if directed to the opposite (non-coding) strand, they can also introduce G→A coding changes. These represent just 2 out of 12 possible types of base edits. In principle, the existence of adenine deaminase enzymes that convert adenine to inosine, which is then replaced with guanine, would permit A→G edits (resulting in either A→G or T→C coding changes, depending on which strand was targeted). Unfortunately, no natural adenine deaminases that act on DNA are known to exist. However, by fusing *Escherichia coli* adenine tRNA deaminase TadA (ecTadA) to dCas9, Gaudelli *et al* hoped to demonstrate adenine base editing capability on targeted DNA sequences<sup>[9]</sup>. Mutagenesis of the ecTadA domain in conjunction with selection

for editing activity revealed that A106V and D108N mutations yielded a base editor capable of editing adenine to guanine in DNA. Successive rounds of mutagenesis, selection, and additional improvements yielded ABE7.10 (**Fig. 2**), an A→G base editor capable of reaching up to 50% efficiency in human cells with low indel rates<sup>[9]</sup>. The editing window ranges from positions 4 to 7 in the protospacer sequence for ABE7.10 to positions 4 to 9 with earlier versions of adenine base editors (ABE7.9, ABE7.8, and ABE6.3). Off-target activity by ABE7.10, ABE7.9, and ABE 7.8 in HEK 293T cells appeared to be lower than that caused by standard Cas9 editing, and no ABE-induced editing was detected outside on-target or off-target protospacers.

Koblan *et al* improved the efficiency of ABE7.10 through modification of nuclear localization signals



**Fig. 2 Schematic of mechanism of action of ABE7.10, an adenine base editor.** A: adenine; C: cytosine; G: guanine; I: inosine/hypoxanthine; T: thymine; N: any base

and codon optimization, yielding a version called ABEmax; a similar approach improved the efficiency of the cytosine base editor BE4<sup>[10]</sup>. Huang *et al* performed further development of both adenine and cytosine base editors to use alternative PAMs and to expand their editing windows, thereby increasing their targeting range<sup>[11]</sup>.

### Off-target effects of base editors

With standard CRISPR-Cas9 genome editing, off-target mutagenesis—*i.e.*, edits at genomic sites other than the intended target site—can occur at sites that share a high degree of sequence similarity with the protospacer sequence encoded in the gRNA. The same has proven to be true of base editors<sup>[12–14]</sup>, although comparisons of Cas9, cytosine base editors, and adenine base editors using the same gRNAs have shown distinct off-target profiles.

Recently, a variety of studies have raised concern about gRNA-independent off-target base editing, caused by the deaminase domain acting in isolation (without the need for engagement of DNA by the Cas9-gRNA complex). Zuo *et al* compared off-target single nucleotide variants (SNVs) caused by Cas9, BE3, and ABE 7.10<sup>[15]</sup>. They injected one cell of a two-cell mouse embryo with one of the genome-editing tools, and after growth of the embryo, they directly compared SNV frequencies between edited and nonedited embryonic cells on embryonic day 14.5. The excess of SNVs in the edited cells was attributed to the genome-editing tool. Although embryos injected with Cas9 or ABE7.10 showed induced SNV rates similar to that of control embryos, embryos injected with BE3 had an SNV rate approximately twenty times higher than that of the control group (243 SNVs per embryo). Approximately 90% of all BE3-associated SNVs were C→T mutations; this bias was not observed in the Cas9 or ABE7.10 experimental groups. SNV formation in the BE3 group was gRNA-independent, *i.e.*, was not linked to protospacer sequence similarity or the presence of PAMs. As a result, the authors hypothesized that the off-target effects were caused by the overexpression of the APOBEC1 subunit. Similar results were found by Jin *et al* for cytosine base editing in rice<sup>[16]</sup>.

Studies by Grünwald *et al*<sup>[17–18]</sup> and Zhou *et al*<sup>[19]</sup> showed that the gRNA-independent off-target effects are not limited to DNA. RNA sequencing of cells treated with either cytosine base editors or adenine base editors revealed transcriptome-wide off-target editing of RNA. Although the same groups showed

that protein engineering of base editors, specifically in the deaminase domain, can reduce gRNA-independent off-target effects on RNA and DNA, the consequences of these off-target effects require further scrutiny, particularly if base editors are to be used for therapeutic purposes.

### Potential use of base editors for mutagenesis screens

Although the base editors were intended to be used in a sequence-specific manner, other base-editing strategies exist to create a library of point mutations in a defined location on a genome. Targeted AID-mediated mutagenesis (TAM), developed by Ma *et al*, uses a dCas9 fused to an AID in conjunction with gRNAs to mutate cytosine to the other three bases in loci targeted by the gRNAs<sup>[20]</sup>. After removing the nuclear export sequence from AID to generate AIDx, over 20% of C bases (or G bases, if targeting the opposite, non-coding strand) in the targeted GFP locus could be modified to another base using TAM. Some bias for C→T or G→A mutations was observed over the other possible base mutations, and a small proportion of A and T bases underwent mutagenesis at the targeted locus. When TAM was applied in the presence of a uracil glycosylase inhibitor, mutation frequencies increased; however, editing became strictly limited to C→T mutations between 12<sup>th</sup> and 16<sup>th</sup> nucleotides upstream of the PAM sequence.

Hess *et al* detailed an alternative approach towards library creation through the editing strategy called CRISPR-X<sup>[21]</sup>. By using dCas9 in conjunction with gRNAs bearing MS2 hairpin binding sites, they were able to recruit AID variants fused to MS2 binding protein to mutagenize longer stretches of DNA, rather than being restricted to small nucleotide windows in the protospacer. In particular, one hyperactive variant of AID, AID\*Δ, was able to edit bases in GFP between 50 nucleotides upstream and 50 nucleotides downstream of the PAM sequence. Similar to TAM, CRISPR-X showed a preference for C→T and G→A mutations and demonstrated a more limited capacity for A and T base editing.

### Potential use of base editors for therapeutic applications

The ability to edit bases directly allows for the disruption of genes *in vivo* without the need to create DSBs. For example, Chadwick *et al* used cytosine base editing to introduce nonsense mutations into the *Pcsk9* gene in adult mice<sup>[22]</sup>. Delivery of BE3 and a

gRNA *via* adenoviral vector into the liver caused a reduction in plasma PCSK9 levels by 56%. Furthermore, cholesterol levels in the base-edited mice were reduced by roughly 30%. In subsequent work, cytosine base editing to disrupt the *Angptl3* gene in adult mice resulted in substantially reduced blood cholesterol and triglyceride levels<sup>[23]</sup>, and cytosine base editing to disrupt the *Pcsk9* gene or the *Hpd* gene in fetal mice resulted in postnatal reduction of cholesterol levels or cure of the disease hereditary tyrosinemia type 1<sup>[24]</sup>.

Correction of disease-causing mutations *via* precision editing with standard Cas9 genome editing has largely required HDR. Since HDR is limited to cells in the S or G2 phase of mitosis, precision editing of non-mitotic cells is difficult. However, base editors are not reliant on HDR; as demonstrated by Yeh *et al*, the editing of postmitotic cochlear cells in mice is feasible with cytosine base editor BE3<sup>[25]</sup>. By injecting BE3 and a gRNA in the form of a preassembled ribonucleoprotein *via* cationic liposomes, serine-33 in  $\beta$ -catenin was edited to phenylalanine (TCT codon edited to TTT), allowing for the transdifferentiation of supporting cells into hair cells. Base editing of cochlear tissue was confirmed *via* sequencing, showing an editing rate between 0.7% and 3.0% depending on the region of the cochlea. In contrast, standard Cas9 editing *via* HDR showed negligible signs of efficacy in cochlear cells. In subsequent work, Villiger *et al* used a variant of SaBE3, delivered into the liver *via* adeno-associated viral (AAV) vectors, to directly correct a pathogenic T→C mutation in the *Pah* gene with an editing rate as high as 29% and thereby treat the disease phenylketonuria in adult mice<sup>[26]</sup>.

Adenine base editing has also been demonstrated to generate mutations in mice. Ryu *et al* used ABE7.10 to correct a nonsense mutation causing Duchenne muscular dystrophy in *Dmd* mutant mice<sup>[27]</sup>. In this study, a dual trans-splicing AAV strategy was used to deliver ABE7.10 into the muscle. Intramuscular injection repaired the nonsense mutation with a 3.3% correction rate, restoring dystrophin production in around 17% of myofibers. In a more recent study, Wu *et al* delivered ABE6.3 *via* tail-vein hydrodynamic injection into mice to correct a point mutation that causes exon skipping in the fumarylacetoacetate hydrolase (*Fah*) gene, thereby ameliorating a mouse model for hereditary tyrosinemia type 1<sup>[28]</sup>. An editing efficiency of around 9.5% was observed in hepatocytes 32 days after treatment; no significant indel rate was detected. Codon-optimized ABE6.3 (RA6.3) was delivered in lipid nanoparticles *via* the

tail vein in a separate experiment; editing rates of around 0.4% were observed<sup>[28]</sup>.

The use of base editors to correct deleterious mutations in human embryos has been explored. Liang *et al* used BE3 to correct *HBB* -28, an A→G mutation causing  $\beta$ -thalassemia<sup>[29]</sup>. By injecting BE3 mRNA and gRNA directly into embryos, they were able to achieve substantial base editing efficiencies at the targeted region. In 9 out of 22 tested embryos, a G→A correction was observed for *HBB* -28; in a single embryo, a G→C edit was observed instead.

### Practical example—"reversible" base editing

To demonstrate the utility of the various base editors that are now available, we used both a cytosine base editor and an adenine base editor to explore the feasibility of treating patients with the disease hereditary tyrosinemia type 1 (HT1) caused by a specific founder mutation. HT1 is an autosomal recessive disorder caused by a deficiency of FAH, resulting in the inability of hepatocytes to properly catabolize the amino acid tyrosine and in the accumulation of toxic intermediate metabolites<sup>[30]</sup>. In the absence of treatment, HT1 can result in fulminant liver failure and death soon after birth. The *FAH* c.1062+5G>A splice site mutation has an estimated carrier rate as high as 1 in 20 people in the Saguenay-Lac-Saint-Jean region of Québec, Canada, and due to the fact that more than 1 in 2000 newborns in that region had the disease, universal newborn screening for HT1 was instituted in Québec in 1970<sup>[31]</sup>.

Because of the potential to directly correct the pathogenic G→A splice site mutation with an adenine base editor, we sought to test the efficacy of base editing against the mutant sequence. However, no human-derived cells with the *FAH* c.1062+5G>A splice site mutation were available. We noted that cytosine base editing with BE3 using a gRNA oriented towards a nearby PAM placed the site of the mutation within BE3's editing window, at position 5 in the protospacer sequence (**Fig. 3**). Thus, we first sought to use BE3 to introduce a C→T edit on the opposite strand (resulting in the precise G→A splice site mutation on the coding strand) in HEK 293T cells.

We transfected plasmids encoding BE3 (pCMV-BE3, a gift from David Liu, Addgene plasmid No. 73021) and the appropriate gRNA (protospacer+PAM= 5'-GATACTCACCGGCCCGCTGA-3' +5'-TGG-3') into cells and clonally expanded single cells a week following transfection. Out of approximately 20 clonal cell lines, only one displayed base editing at the target



FAH sequence	Allele	Frequency
ACCATCAGCGGGCCGGT <b><u>G</u></b> ATCTGGCTGCACTGAGGGC	Wild-type	
ACCATCAGCGGGCCGGT <b><u>A</u></b> ATCTGGCTGCACTGAGGGC	Quebec	
<b>ACCA</b> TCAGCGGGCCGGT <b><u>G</u></b> ATCTGGCTGCACTGAGGGC	Wild-type	
ACCATCAGCGGGCCGGT <b><u>AA</u></b> AATCTGGCTGCACTGAGGGC	293.BE3.1	
ACCATCAGCGGGCCGGT <b><u>ATA</u></b> AATCTGGCTGCACTGAGGGC	293.BE3.2	
ACCATCAGCGGGCCGGT <b><u>ATT</u></b> AATCTGGCTGCACTGAGGGC	293.BE3.3	
ACCATCAGCGGGCCGGT <b><u>ATA</u></b> AATCTGGCTGCACTG <b><u>AG</u></b> GGC	293.BE3.1	
.....GTA <b><u>A</u></b> GTGTC.....	ABE edit	5.1%
.....GT <b><u>G</u></b> AGTGTC.....	ABE edit	1.9%
.....GT <b><u>AG</u></b> GTGTC.....	ABE edit	0.9%
.....GT <b><u>GG</u></b> GTGTC.....	ABE edit	0.8%
.....GTA <b><u>A</u></b> GTATC.....	ABE edit	0.1%

**Fig. 3** Reversible base editing of a prevalent mutation responsible for hereditary tyrosinemia type 1 (HT1). The wild-type and "Quebec" alleles (FAH c.1062+5G>A splice site mutation) differ by a G→A change (bold and underlined). Cytosine base editing with BE3 (protospacer in box, PAM in pink, editing window in blue) generated three different alleles in a HEK 293T clonal cell line, two alleles of which have the Quebec G→A mutation (bold and underlined; other changes are only in bold), designated 293.BE3.1 and 293.BE3.2. Adenine base editing with ABE7.10 (protospacer in box, PAM in pink, editing window in blue) and a gRNA perfectly matched to the 293.BE3.1 allele resulted in ≈9% of the 293.BE3.1 alleles in the treated cells having reversion of the Quebec G→A mutation to the wild-type G.

site. We expanded this cell line and again performed single cell cloning to ensure a "clean" clonal cell line. Upon deep sequencing of the target site in the resulting cell line, we observed that there were three distinct alleles (indicating that the cells were triploid for the chromosomal region in question, not surprising given the cells' immortalized state), none of them wild-type (**Fig. 3**). In one allele (293.BE3.1), the desired c.1062+5G>A mutation was present, along with an additional upstream G→A edit (position 7 in the protospacer sequence). In the second allele (293.BE3.2), the desired c.1062+5G>A mutation was present, along with two additional upstream G→A edits (positions 7 and 9 in the protospacer sequence). In the third allele (293.BE3.3), the site of the desired mutation instead harbored a G→T edit, along with one additional upstream G→A edit (position 7 in the protospacer sequence). Of note, the additional upstream G→A edits were at positions lying within or close to BE3's predicted editing window.

This exercise confirmed two known shortcomings of base editing. First, all of the potentially editable bases within the base editor's window can be edited, which can make it prohibitive to obtain alleles with just the desired base edit. This occurred in this example, where none of the alleles had only the desired c.1062+5G>A mutation. Second, the edits might not always have the expected outcomes. In this example, one of the alleles had a noncanonical G→T edit rather the desired (and likeliest) G→A edit.

Given the difficulty in obtaining a cell line with just the desired c.1062+5G>A mutation, we proceeded to

use the triple-edited-allele cell line to assess whether adenine base editing of the mutation with ABE7.10 was feasible. We designed a gRNA oriented towards a nearby PAM whose protospacer matched only the first of the above-described three edited alleles (c.1062+5G>A mutation and one additional upstream G→A edit; designated 293.BE3.1), which should have the effect of constraining the activity of ABE7.10 to just that one allele and sparing the other alleles. The PAM placed the site of the c.1062+5G>A mutation within ABE7.10's editing window, at position 5 in the protospacer sequence (**Fig. 3**).

We transfected plasmids encoding ABE7.10 (pCMV-ABE7.10, a gift from David Liu, Addgene plasmid No. 102919) and the appropriate gRNA (protospacer+PAM=5'-GT**AA**AATATCTGGCTGCAC-TG-3'+5'-AGG-3'; deviation from wild-type sequence in bold and underlined) into the cell line. One week following transfection, we harvested genomic DNA from the cells and performed deep sequencing of the target site. Our previously described algorithm<sup>[12]</sup> identified the sequence reads from the first of the above-described three edited alleles (293.BE3.1) and discarded the other reads. The algorithm then determined the proportion of the selected reads in which the c.1062+5G>A mutation had been successfully corrected. We found that about 9% of the reads were corrected at that specific position (**Fig. 3**). However, we found that in almost all of the corrected reads, additional adenines had been converted to guanine (every possible combination of positions 3, 4, and 7 in the protospacer sequence). In a small

proportion of the corrected reads only the c.1062+5G>A mutation was edited to guanine, again highlighting the challenging of restricting the editing activity of a base editor to just the desired position. Of note, we did observe that none of the reads harbored indel mutations.

Despite the inherent challenges, we were able to use a cytosine base editor and an adenine base editor to serially install and then correct a real-life pathogenic mutation in human cells. To our knowledge, this is one of the first reported demonstrations of "reversible" base editing. We note that HT1 is a disease in which any successfully corrected hepatocytes will have a selective advantage over uncorrected cells, and even a small proportion of corrected hepatocytes can ultimately reconstitute the entire liver and potentially cure the disease<sup>[24]</sup>. Thus, the obstacle presented by undesired additional A→G edits in the vicinity of a corrected c.1062+5G>A mutation is not insuperable from the perspective of therapeutic base editing for HT1. Nonetheless, as noted earlier, alteration of the Cas9 component of a base editor can serve to narrow the width of the editing window, and a suitably tailored variant of ABE7.10 might therefore achieve highly specific correction of only the c.1062+5G>A mutation.

## Conclusions

Although the "CRISPR craze" that has taken the biomedical community by storm since early 2013 has captured the attention of not just scientists but the lay public, in fact it is base editing that might ultimately prove to be the truly revolutionary technology, as it permits precision editing in a manner that goes far beyond the capacity of standard CRISPR-Cas9 and previous generations of genome-editing tools, all of which have relied on DSBs and the difficult-to-harness consequences of DSB repair. The ability to introduce or correct specific mutations—or both, which could permit reversible editing—with reasonably high efficiency, as demonstrated in the first wave of papers on base editing described here, as well as in the practical example presented here, portends a new therapeutic approach that might ultimately surpass standard genome editing. The coming years will undoubtedly see the development and refinement of base-editing tools as well as new approaches such as the recently described "prime editing"<sup>[32]</sup> that will open up the entire genome to precision editing.

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## References

- [1] Sander JD, Joung JK. CRISPR-Cas systems for editing, regulating and targeting genomes[J]. *Nat Biotechnol*, 2014, 32(4): 347–355.
- [2] Adli M. The CRISPR tool kit for genome editing and beyond[J]. *Nat Commun*, 2018, 9(1): 1911.
- [3] Komor AC, Kim YB, Packer MS, et al. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage[J]. *Nature*, 2016, 533(7603): 420–424.
- [4] Rees HA, Komor AC, Yeh WH, et al. Improving the DNA specificity and applicability of base editing through protein engineering and protein delivery[J]. *Nat Commun*, 2017, 8(1): 15790.
- [5] Kim YB, Komor AC, Levy JM, et al. Increasing the genome-targeting scope and precision of base editing with engineered Cas9-cytidine deaminase fusions[J]. *Nat Biotechnol*, 2017, 35(4): 371–376.
- [6] Nishida K, Arazoe T, Yachie N, et al. Targeted nucleotide editing using hybrid prokaryotic and vertebrate adaptive immune systems[J]. *Science*, 2016, 353(6305): aaf8729.
- [7] Li XS, Wang Y, Liu YJ, et al. Base editing with a Cpf1-cytidine deaminase fusion[J]. *Nat Biotechnol*, 2018, 36(4): 324–327.
- [8] Komor AC, Zhao KT, Packer MS, et al. Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity[J]. *Sci Adv*, 2017, 3(8): eaao4774.
- [9] Gaudelli NM, Komor AC, Rees HA, et al. Programmable base editing of A·T to G·C in genomic DNA without DNA cleavage[J]. *Nature*, 2017, 551(7681): 464–471.
- [10] Koblan LW, Doman JL, Wilson C, et al. Improving cytidine and adenine base editors by expression optimization and ancestral reconstruction[J]. *Nat Biotechnol*, 2018, 36(9): 843–846.
- [11] Huang TP, Zhao KT, Miller SM, et al. Circularly permuted and PAM-modified Cas9 variants broaden the targeting scope of base editors[J]. *Nat Biotechnol*, 2019, 37(6): 626–631.
- [12] Kim D, Lim K, Kim ST, et al. Genome-wide target specificities of CRISPR RNA-guided programmable deaminases[J]. *Nat Biotechnol*, 2017, 35(5): 475–480.
- [13] Liang PP, Xie XW, Zhi SY, et al. Genome-wide profiling of adenine base editor specificity by EndoV-seq[J]. *Nat Commun*, 2019, 10(1): 67.
- [14] Kim D, Kim DE, Lee G, et al. Genome-wide target specificity of CRISPR RNA-guided adenine base editors[J]. *Nat Biotechnol*, 2019, 37(4): 430–435.
- [15] Zuo EW, Sun YD, Wei W, et al. Cytosine base editor generates substantial off-target single-nucleotide variants in mouse

- embryos[J]. *Science*, 2019, 364(6437): 289–292.
- [16] Jin S, Zong Y, Gao Q, et al. Cytosine, but not adenine, base editors induce genome-wide off-target mutations in rice[J]. *Science*, 2019, 364(6437): 292–295.
- [17] Grünewald J, Zhou RH, Garcia SP, et al. Transcriptome-wide off-target RNA editing induced by CRISPR-guided DNA base editors[J]. *Nature*, 2019, 569(7756): 433–437.
- [18] Grünewald J, Zhou RH, Iyer S, et al. CRISPR DNA base editors with reduced RNA off-target and self-editing activities[J]. *Nat Biotechnol*, 2019, 37(9): 1041–1048.
- [19] Zhou CY, Sun YD, Yan R, et al. Off-target RNA mutation induced by DNA base editing and its elimination by mutagenesis[J]. *Nature*, 2019, 571(7764): 275–278.
- [20] Ma YQ, Zhang JY, Yin WJ, et al. Targeted AID-mediated mutagenesis (TAM) enables efficient genomic diversification in mammalian cells[J]. *Nat Methods*, 2016, 13(12): 1029–1035.
- [21] Hess GT, Frésard L, Han K, et al. Directed evolution using dCas9-targeted somatic hypermutation in mammalian cells[J]. *Nat Methods*, 2016, 13(12): 1036–1042.
- [22] Chadwick AC, Wang X, Musunuru K. *In vivo* base editing of PCSK9 (proprotein convertase subtilisin/kexin type 9) as a therapeutic alternative to genome editing[J]. *Arterioscler Thromb Vasc Biol*, 2017, 37(9): 1741–1747.
- [23] Chadwick AC, Evitt NH, Lv WJ, et al. Reduced blood lipid levels with *in vivo* CRISPR-Cas9 base editing of ANGPTL3[J]. *Circulation*, 2018, 137(9): 975–977.
- [24] Rossidis AC, Stratigis JD, Chadwick AC, et al. In utero CRISPR-mediated therapeutic editing of metabolic genes[J]. *Nat Med*, 2018, 24(10): 1513–1518.
- [25] Yeh WH, Chiang H, Rees HA, et al. *In vivo* base editing of post-mitotic sensory cells[J]. *Nat Commun*, 2018, 9(1): 2184.
- [26] Villiger L, Grisch-Chan HM, Lindsay H, et al. Treatment of a metabolic liver disease by *in vivo* genome base editing in adult mice[J]. *Nat Med*, 2018, 24(10): 1519–1525.
- [27] Ryu SM, Koo T, Kim K, et al. Adenine base editing in mouse embryos and an adult mouse model of Duchenne muscular dystrophy[J]. *Nat Biotechnol*, 2018, 36(6): 536–539.
- [28] Song CQ, Jiang TT, Richter M, et al. Adenine base editing in an adult mouse model of tyrosinaemia[J]. *Nat Biomed Eng*, 2020, 4(1): 125–130.
- [29] Liang PP, Ding CH, Sun HW, et al. Correction of  $\beta$ -thalassemia mutant by base editor in human embryos[J]. *Protein Cell*, 2017, 8(11): 811–822.
- [30] Morrow G, Tanguay RM. Biochemical and clinical aspects of hereditary tyrosinemia type 1[M]//Tanguay RM. Hereditary Tyrosinemia: Pathogenesis, Screening and Management. Cham: Springer, 2017: 9–21.
- [31] De Braekeleer M, Larochelle J. Genetic epidemiology of hereditary tyrosinemia in Quebec and in Saguenay-Lac-St-Jean[J]. *Am J Hum Genet*, 1990, 47(2): 302–307.
- [32] Anzalone AV, Randolph PB, Davis JR, et al. Search-and-replace genome editing without double-strand breaks or donor DNA[J]. *Nature*, 2019, 576(7785): 149–157.

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