Base Editing: A Promising Endeavour in Gene Therapy

Dr. Archana Kumari Srivastava[1], Senior Executive; Mr. Vijayanand K[1], Deputy General Manager; Mr. Sudip Kumar Majumder[1], Vice President

[1]Department of Biotechnology, Jodas Expoim Pvt. Ltd

Base Editing Therapy is a technology that introduces single-nucleotide variants (SNVs) precisely and efficiently at targeted genomic sequences without causing double-stranded breaks in the DNA enabling it as an efficient technique of genome editing (Figure 1). Nearly half of known pathogenic genetic variants are due to SNVs and base editing therapy holds enormous potential for the treatment of these genetic disorders by either temporary RNA or permanent DNA base alterations. Correction of single point mutations will be a major point of interest in the upcoming times for the scientific community for precision medicine.

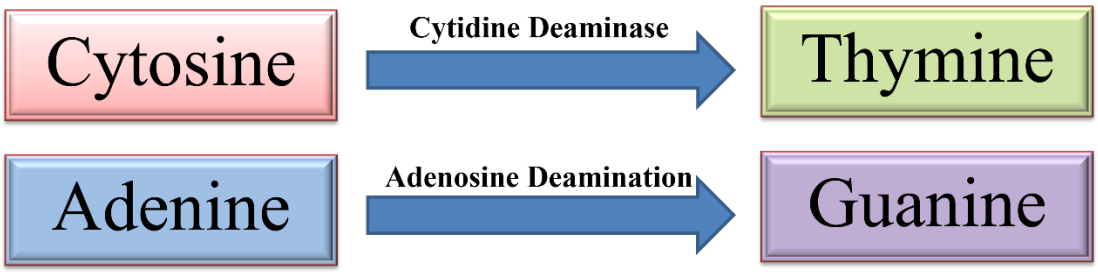

Figure 1: Base editor technology chemically modifies Cytosine to Thymine and Adenine to Guanine

The concept of Genome editing was originally derived from clustered regularly interspaced short palindromic repeat (CRISPR), a naturally occurring bacterial and archaeal defence against invading viruses by inducing double-stranded DNA breaks (DSBs) or RNA cleavage. This technology utilizes CRISPR-associated proteins, Cas9 and Cas12, typically repaired through non-homologous end joining (NHEJ) or homology-directed repair (HDR). NHEJ-resolved DSBs result in non-specific insertions or deletions (indels) at the site of the DSB, often resulting in frameshifts and gene knockout. Unfortunately, DSBs can be deleterious for the target cells. Here comes the role of Base Editor (which includes a CRISPR protein bound to a guide RNA and a base editing deaminase enzyme) that solves this problem by carrying out desired chemical modification of the targeted DNA base and avoids nucleic acid backbone cleavage.

Whereas the origins of base editing technology begin decades ago with RNA base editors, both categories have recently seen an explosion in development. Before moving ahead to understand base editing few terminologies needs to be mentioned.

Guide RNA (gRNA): A short stretch of RNA that recognizes the target DNA region of interest and directs the Cas enzyme to bind for editing to occur often known as the spacer and single guide RNA.

Protospacer: A DNA locus of interest targeted with genome editing agent; base pairs with the guide RNA.

Protospacer adjacent motif (PAM): A variable region on the 5′or 3′end of the protospacer, required for Cas protein binding to the target locus.

R-loop: A tripartite structure consisting of unpaired DNA and a paired DNA: RNA hybrid following R-loop formation, the unpaired or single-stranded DNA is accessible for base editing.

Classes of Base Editors

Base editors can be sorted into two main categories:

1. DNA targeting base editors
2. RNA targeting base editors

1. **DNA Base editors -** It is of two types as described below

   a. **Cytosine base editors (CBEs)**

CBE was first DNA base editor developed which can edit genome without using DSBs. A naturally occurring **cytidine deaminase** enzyme was used to convert target cytosines to uracil, which has the base pairing properties of thymine. This was expected to catalyse an overall C•G to T•A base pair conversion following the cell's use of uracil as a template for repair. The mechanism is explained in Figure 2.

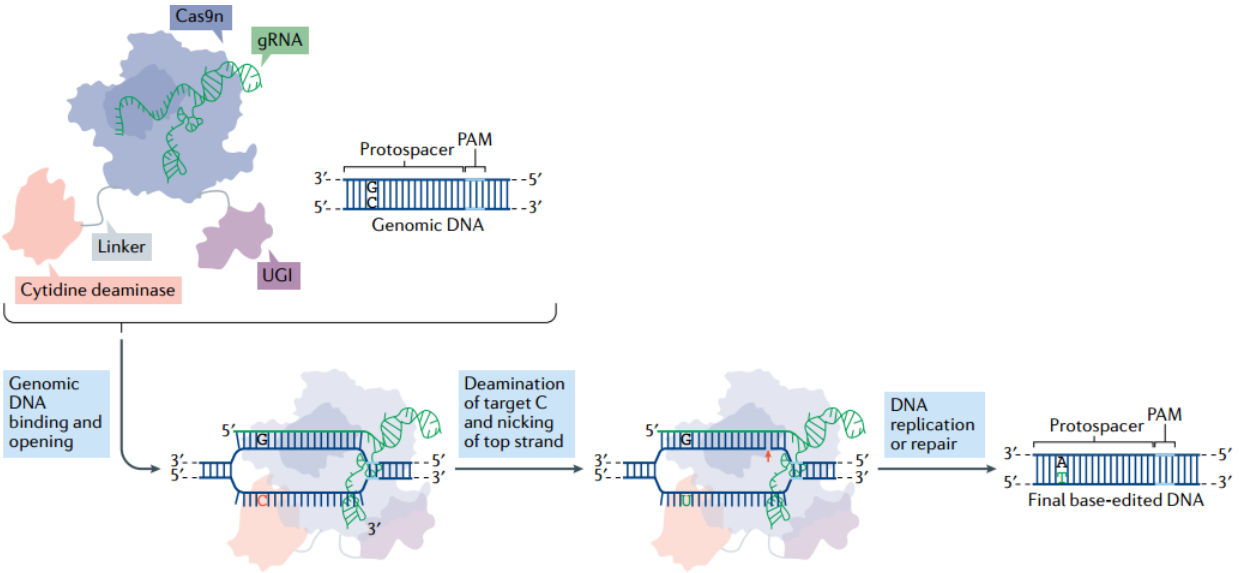

Figure 2: Cytosine base editor (CBE) mechanism.

Principle components of the CBE are designated in coloured text boxes. If uracil glycosylase inhibitor (UGI) is present (an optional component), it will 'protect' the U-G intermediate from excision by uracil DNA glycosylase (UDG) to boost efficiency of the final base edited DNA outcome. The nickase version of Cas9 (Cas9n) nicks the top strand (red arrow) whereas the cytidine deaminase converts cytosine (red) to uracil (green).

Source: Porto et al., Base editing: advances and therapeutic opportunities Nature Review Drug Discovery. 2020 19(12):839-859.

b. Adenine base editors (ABEs)

Drawing inspiration from CBEs, adenosine deamination chemistry were identified which would lead inosine to be read as guanine by replication and transcription machinery. Naturally occurring adenosine and adenine deaminase enzymes do exist, but their substrates are confined to various forms of RNA. In spite of having numerous naturally occurring adenosine deaminases such as *Escherichia coli*TadA (ecTadA), human ADAR2, mouse ADA and human ADAT but none gave required level yield of A•T to G•C base editing. Hence, after several round of mutations in natural adenosine deaminases desired enzyme from ecTadA was evolved. Unlike CBE, no DNA repair manipulation component (such as UGI) is required due to the rare nature of the inosine intermediate. Figure 3 demonstrates adenine base editor (ABE) mechanism.

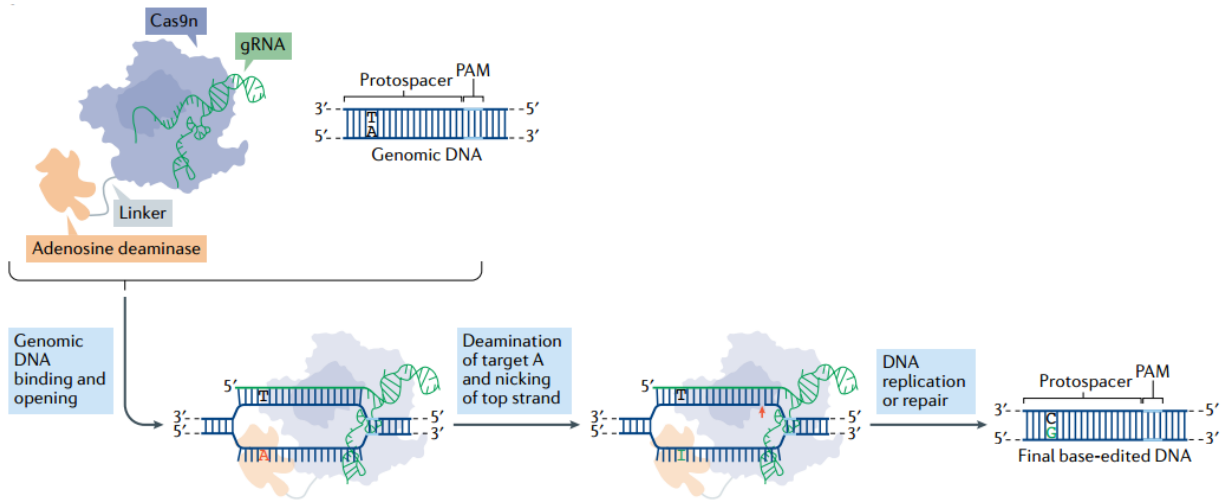

Figure 3: Adenine base editor (ABE) mechanism.

Through ABE-mediated editing, an A•T to G•C base pair conversion is achieved via an inosine-containing intermediate.

Source: Porto et al., Base editing: advances and therapeutic opportunities Nature Review Drug Discovery. 2020 19(12):839-859.

2. RNA Base Editors- RNA base editors are classified according to the modification that they introduce. In contrast to DNA base editing which introduces permanent changes in the genome, RNA base editing offers reversible modifications to a cell's genetic material.

a. A-to-I RNA base editors

Chronologically, RNA base editing predates both DNA base editing and the use of CRISPR for genome editing purposes. Around 25 years ago adenosine to inosine (A-to-I) RNA base

editing was explored, when the Adenosine Deaminase Acting on RNA (ADAR) enzyme was used to correct a premature stop codon in a synthetic mRNA construct. Later on ADARs were tethered to antisense oligonucleotides (ASOs), allowing for targeted, precise A-to-I editing in live cells. Base pairing between the ASO and the target RNA transcript increase the specificity of the deaminase domain of ADAR (ADARDD), particularly when the target adenosine is embedded within a bulged A•C mismatch (Figure 4).

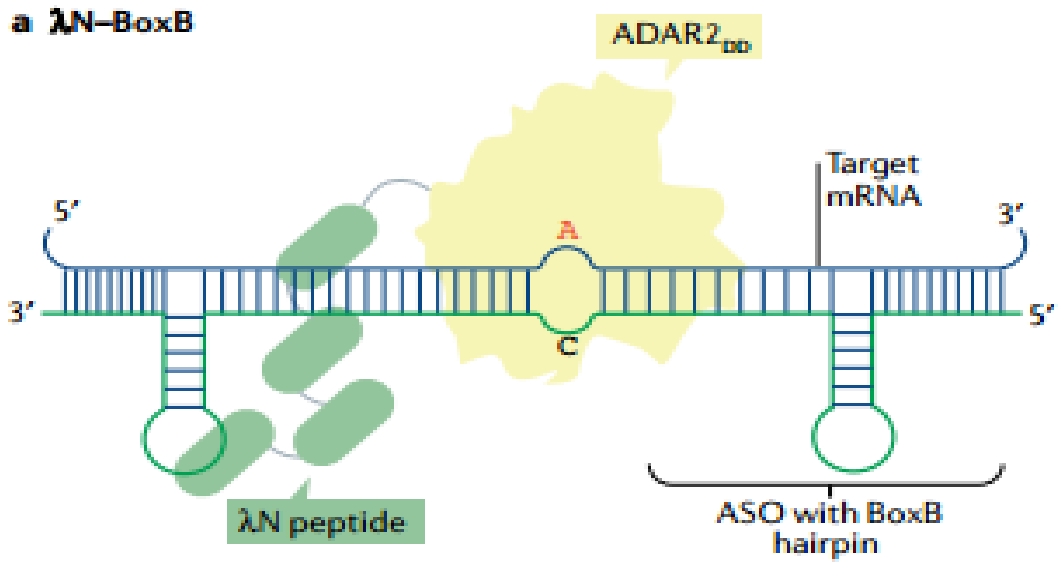

Figure 4: Illustration of A-to-I RNA Base Editor

Source: Porto et al., Base editing: advances and therapeutic opportunities Nature Review Drug Discovery. 2020 19(12):839-859.

b. C-to-U RNA base editors

Even though RNA cytosine deaminase enzymes naturally exist, their high activity for any cytosine present in single-stranded RNA has barred their use for strategizing precise RNA base editors. RESCUE (RNA editing for specific C-to-U exchange) has been developed to meeting this challenge. Base editing in RNA is diagrammatically explained in Figure 5.

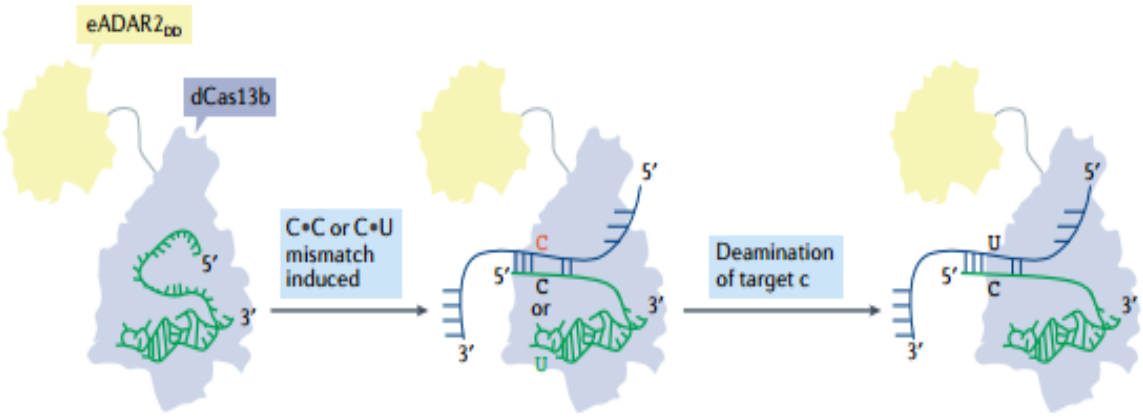

Figure 5: Diagrammatic representation of C-to-U RNA Base editing Mechanism

Source: Porto et al., Base editing: advances and therapeutic opportunities Nature Review Drug Discovery. 2020 19(12):839-859.

FDA Approved Base Editing Therapy

Drug Name	Indications	Base Editing	Status
BEAM-201	Used for the treatment of relapsed/refractory T-cell acute lymphoblastic leukemia (T-ALL)/T-cell lymphoblastic lymphoma (T-LL). BEAM-201 is a potent and specific anti-CD7, multiplex-edited, allogeneic chimeric antigen receptor T-cell development candidate.	T cells from healthy donors are base edited at the same time at 4 genomic loci. Then, they are transduced with a lentivirus coding for an anti-CD7 CAR.	U.S. Food and Drug Administration (FDA) has lifted the clinical hold and cleared the Investigational New Drug (IND) application for BEAM-201

Base Editing Therapy in Pipeline

Drug Name	Indications	Base Editing	Status
BEAM-101	BEAM-101 is a patient-specific, autologous haematopoietic cell therapy designed as a one-time treatment for sickle cell disease (SCD) and beta-thalassemia (BT)	It is an adenine base editor (ABE) that reproduces single base changes observed in individuals with hereditary persistence of fetal hemoglobin, or HPFH, in which elevated levels of fetal hemoglobin protect these individuals from the effects of sickle cell disease or beta-thalassemia	Phase I Clinical Trial

BEAM-102	Used for Sickle Cell Disease	BEAM-102 is an ABE that directly corrects the causative mutation in sickle cell disease, converting it into a naturally-occurring human hemoglobin variant, Hb-G Makassar. Individuals with the Makassar variant have normal hematologic parameters and no evidence of hemoglobin polymerization or sickling of red blood cells.	IND (Investigational New Drug) submitted to U.S. FDA
VERVE-101	*In vivo* Base editing therapy to reach the clinic as a potential treatment for Heterozygous Familial Hypercholesterolemia (HeFH)	VERVE-101 consists of an adenine base editor messenger RNA that Verve has licensed from another base editing therapy developer, Beam Therapeutics, as well as an optimized guide RNA targeting the PCSK9 gene packaged in an engineered lipid nanoparticle.	FDA put clinical hold

Manufacturing Steps of Base Editing Therapy

1. Highly efficient Multiplex Base Editing in primary Human T-Cells.
2. Electroporation and Transduction of isolated T-cells with using the Multiplex Base Edited DNA
3. Cryopreservation of Base edited T-cells for infusing to patient.

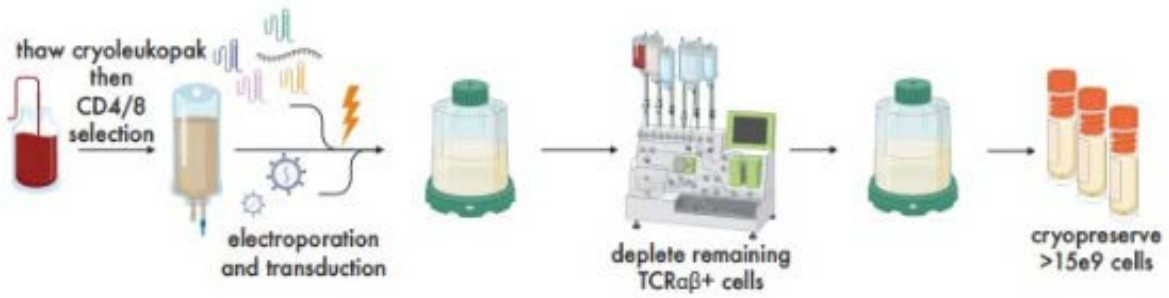

Figure 6: BEAM-201 is produced using a GMP-compliant clinical-scale process that results in the cryopreservation of >15e9 quad-edited cells using healthy donor material isolated from cryoleukopaks ***(Source: Beam Therapeutics)***

Market Valuation of Gene Editing that includes Base Editing Therapy

According to Market research report of Brainy Insights, the global genome editing market was valued at USD 5.89 billion in 2022, increasing at a CAGR of 16.85% from 2022 to 2030 and anticipated to reach USD 20.47 billion by 2030.

Hurdles and Hope associated with Base Editing Therapy

Notable shortcoming of Base Editing is its dependence on suitable PAM adjacent to the target sequence and in a position that places the target bases in the optimal editing window. The primary CBEs and ABEs were designed using the SpCas9n (most common Cas used for genome editing) which is limited to genomic loci containing NGG PAMs. To enhance its array of potential targets, Base Editing harboring orthologous Cas9n or genetically engineered Cas9n variants capable of recognizing non-NGG PAMs has been developed. For some of these enzymes the editing window is shifted or enlarged to enhance target bases that otherwise would be inaccessible due to the lack of an optimal PAM. In addition to that, engineered deaminase variants were also developed for enlargement of the editing window (Antoniou et al., 2021). Base editing is one of the most recent chapters added in the field genetic engineering and gene therapy and it is still in its nascent stage but in the upcoming time it could be one of the most effective tools to resolve genetic disorders.

References:

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