Base Editing, the Story So Far

Gene editing technologies have been evolving exponentially since CRISPR was first commercialised in early 2012. The recent emergence of CRISPR-based base editing platforms has attracted attention in cell and gene therapy fields as a gene editing technology that could drive the evolution of new therapeutic applications. This article will touch upon the potential therapeutic applications of base editing and explain why there is so much hype around this latest incarnation of CRISPR-based gene editing.

With the rapid emergence of CRISPR-Cas gene editing over the past decade, the field of genome engineering has taken a major step forward in its ability to support both cell and gene therapy. The transition from initial gene editing applications in mammalian cells[1,2] to the US Food and Drug Administration's (FDA) investigational new drug (IND) for CRISPR-engineered cell-based therapies has occurred at an unprecedented rate: the use of CRISPR-based approaches has revolutionised cell-based therapeutics. Expanding on the use of nucleases such as zinc fingers and transcription activator-like effectors, CRISPR-Cas gene editing systems personify the benefit of making efficient and site-specific genomic changes. The advent of base editing has taken this desire to make more precise genomic changes a step further[3,4].

Like all CRISPR-Cas systems, base editing uses a short guide RNA in partnership with a Cas enzyme. For base editing, a nickase version of Cas is used to nick a single strand of DNA along with a deaminase enzyme to enable alteration of a single nucleotide (Figure 1). The use of a nickase as opposed to a nuclease substantially reduces the occurrence of DNA double-strand breaks (DSBs). The power of editing the genome with a high degree of specificity while not causing a DNA DSB is pushing base editing into the therapeutic spotlight. Researchers, clinicians and governing bodies are now looking to harness the potential of base editing as an efficient means for introducing stop codons to disrupt gene function[5], an area that is gaining a lot of traction in the engineering of chimeric antigen receptor (CAR) T cells to target and kill cancer cells.

The Limitations of CRISPR-Cas Gene Editing

RNA-guided Cas enzymes are directed to a DNA target site by a guide RNA leading to the introduction of a DSB into the DNA at that precise location. This break is repaired by the cell's intrinsic DNA repair pathways. Often these repair processes are imprecise, leading to new nucleotide sequences being generated through random insertions and deletions (indels). These indels often produce combinations of missense mutations, nonsense mutations and premature stop codons, which effectively disrupt or knockout the transcription of the gene of interest. However, for clinical applications, the introduction of one or more DSB into the DNA might cause additional safety concerns due to the random nature of indel formation and the possibility of additional DNA breaks occurring elsewhere in the genome with the potential to lead to chromosomal translocations. Additionally, this imprecise nature of indel formation can lead to other undesirable

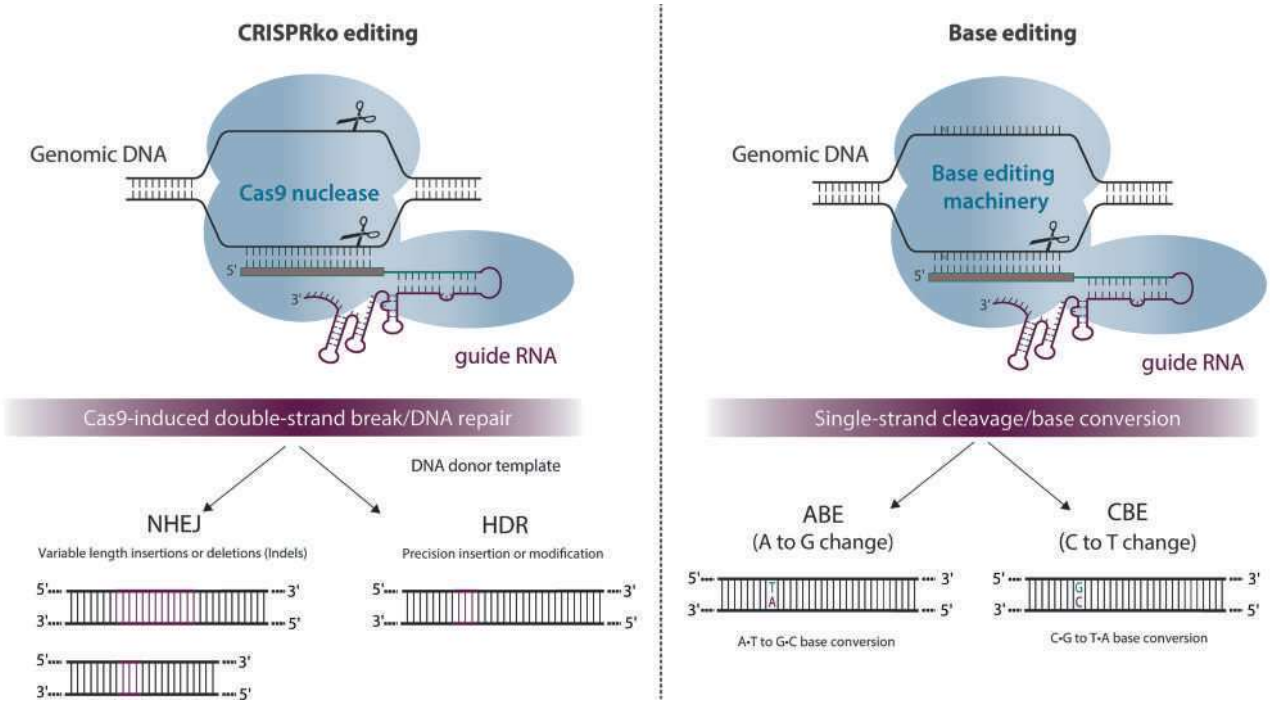

Figure 1. Overview and potential outcomes of CRISPR-Cas9 mediated genome editing and base editing

genomic changes where the downstream impacts are not yet fully characterised. Some of these issues with nuclease-based gene editing have been overcome with carefully selected guide RNAs that do not lead to Cas9-mediated DNA DSBs at sites other than at the gene of interest. However, for some cell therapy applications, multiple genes need to be edited at the same time in the same cell. If standard CRISPR editing platforms are used, then multiple DNA DBSs will be introduced into the genome at the same time, with the potential for chromosomal rearrangements increased. As base editors use a nickase to mediate gene alterations, they could have greater utility in cell therapies where multiple edits are needed to produce the cell-based therapeutic product.

An Emerging Technology

The initial description of a base editing platform was with cytidine base editors (CBEs)[3,4] that enabled recruitment of cytidine deaminase enzymes to a DNA target site via the CRISPR-Cas system, converting C-G base pairs to T-A base pairs, a transition mutation where one pyrimidine base is converted to a different pyrimidine base. These base editors were rapidly followed by adenine base editors (ABEs)[6], which convert A-T base pairs to G-C. More recent systems can convert C-G base pairs to G-C[7,8] and C-G base pairs to A-T[8], demonstrating the capability of base editing to also make transversion mutations, where a pyrimidine base is changed to a purine base (Figure 2). These systems, as well as other novel base editing systems[9], have undergone and continue to undergo phases of evolution and refinement with the goal of providing an efficient, precise, and safe method of changing nucleotide base pairs for therapeutic applications. This ability to make a plethora of both transition and transversion mutations presents an opportunity for highly specific editing of the genome, adding to the therapeutic potential that base editors have.

Therapeutic Potential

Cell-based therapeutics are not a new inception, but their development has been greatly facilitated in recent years by the ability to tailor the genetic make up of a cell using gene engineering. For example, the engineering of T cells to recognise and destroy cancer cells through the introduction of a CAR and deletion of the T cell receptor, has led to new treatments for B cell leukaemias. These CAR-T cell therapies can be classified as either autologous, where a patient's own T cells are removed, engineered, and then infused back into the patient, or allogeneic where a single source of donor cells can be used to create a steady supply of "universal" CAR-T cells to treat multiple patients. While both methods have advantages and disadvantages, they have both had clinical success. In 2017, the FDA approved the use of autologous CAR-T cells for treating B cell lymphoblastic leukaemia, while in 2019 the first allogeneic CAR-T cell approach was approved for investigational use in patients with multiple myeloma.

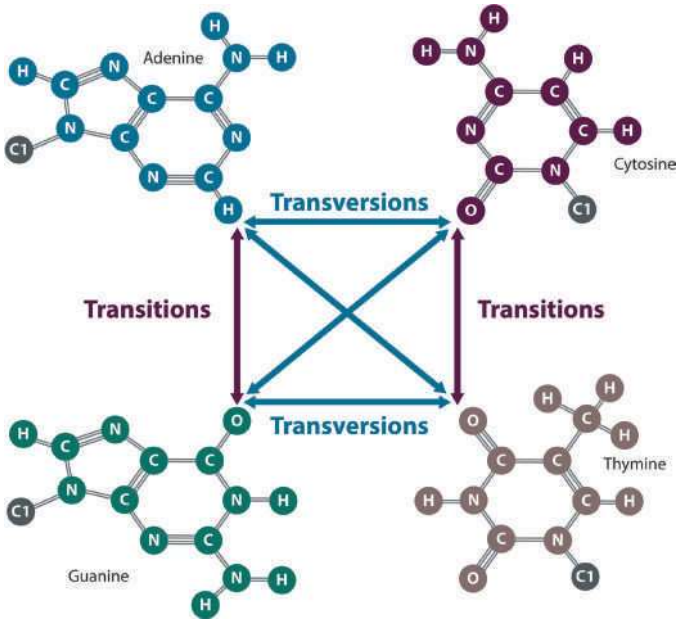

Figure 2. Base editing conversion of transition and transversion nucleotide changes

Despite these successes, CAR-T cells and other CAR-like immune cell-based therapeutics will need to be further modified to treat solid cancers and potentially other human diseases. Some have proposed that CAR-T cells might require upwards of 10 modifications or more to increase their efficacy and longevity, meaning that precision technologies such as base editing could provide a very attractive means to make these multiplex changes.

In 2019, clinical trials were initiated using T cells that had been edited with CRISPR-Cas9 to disrupt the function of three genes. Subsequent analysis of the cells nine months later showed healthy-looking T cells with the CRISPR-Cas9 edited cells persisting and showing few off-target changes[10]. This study offers an important launching point as the field progresses forward with safely editing T cells at multiple sites. It also demonstrates how base editing could offer a compelling route forward. What if T cells and CAR-T cells could be edited precisely without the introduction of a DNA DSB and with much lower levels of indel formation? Base editing has been successfully used for the multiplex knockout of T cell receptor targets[11] and it is only a matter of time before some of these base-editing multiplex approaches advance into the clinic.

On the Horizon

Researchers around the world are working to further characterise and improve base editing technologies as they advance through therapeutic pipelines. Aside from the use of a nickase, base editing can also profit from being similar to but different from standard CRISPR-Cas systems by learning from the methods used to drive the rapid and safe adaption of CRISPR-Cas gene editing in IND-approved therapeutics. Base editing also has a crucial role in treating diseases that arise owing to single point mutations[12,13] emphasising the vast number of therapeutic applications and treatments that could be addressed and expediated by base editing. Point mutations associated with Alzheimer's disease[3], sickle-cell anaemia[6], β-thalassemia[14], and progeria[15] are just a few of the thousands of clinically relevant disease variants in the human genome that have been either corrected or modelled by base editing.

While several questions about base editing remain unanswered, including the efficient DNA-free delivery of this system into primary cells and the major hurdle of *in vivo* delivery for gene therapies as opposed to cell therapies, the

impact that base editing will have on therapeutic development is becoming clear. The exciting precision and efficacy in which base editing can edit the genome is just the start of the base editing story.

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