

Gene Edited iPSCs May Find a Place in Allogeneic Therapies

An Introduction to Pluripotency

Pluripotent: adjective; capable of giving rise to many different cell types. This is the hallmark characteristic of the induced pluripotent stem cell, or iPSC. Since the discovery of reprogramming adult human fibroblasts into an induced state of pluripotency in the early 2000s, the scientific community has been enamored with the possibility of this dynamic cell type.¹ A seemingly vast universe of possibilities exists for the iPSC from the corners of basic research to the depths of clinical medicine.

The pluripotent state has been proven time and again using various protocols for generating cell types and organoids covering nearly every system in the human body. Industry and pharma have also come to favor the iPSC for many of its other qualities. While the iPSC may be considered a “finicky” cell type to work with in the lab, and costly to grow due to reliance on complex growth medias and matrices, it is unique in that it is considered to be a patient (or donor) sourced cell type, but with the added potential for long-term growth in culture. Easily derived from primary skin biopsies or blood, iPSCs maintain the existing genetic and epigenetic makeup of the human from which they came without the ethical dilemmas surrounding the use of embryonic stem cells. Nearly as important as their differentiation potential and proximity to the primary source is the ability of iPSC cultures to be scaled and banked for the purposes of manufacturing gene and cell therapies.

In the undifferentiated state iPSCs have been broadly studied across research fields. They have been pivotal instruments for understanding basic cell biology, advancing drug discovery efforts, and have served as the foundation of clinical therapies. Differentiated but otherwise unmodified iPSCs already began to have a clinical impact within the first decade of their discovery. In 2017, somatic cells harvested from a patient with macular degeneration were converted to iPSCs and differentiated into

retinal pigment epithelial cells, which were then used in the same patient in an effort to restore vision as an autologous (self) cell therapy.² Since then, other clinical uses of autologous stem cell therapies have been investigated for regenerative medicines in the fields of diabetes and cardiology, among others.^{3,4}

Broadening Therapeutic Potential

Today, the use of iPSCs has progressed from regenerative therapies to immunotherapies and other precision medicines. We have already seen the impact of autologous chimeric antigen receptor (CAR) T cells in the clinic where they are used to address hematological malignancies. Autologous therapies avoid concerns over undesired immune response in patients, because typically, even if the cells are engineered *ex vivo* they are still recognised as “self” once returned to the patient’s body. However, engineering any cells *ex vivo* to enable autologous personalised medicine is a time consuming and expensive process because each dose of the therapy must be engineered on-demand for every patient.

Limitations exist even for immunotherapies where both engineered T and natural killer (NK) cells have proven to be robust therapeutic agents. Therapies that rely on autologous primary cells have lengthy production times, sometimes longer than the patient can survive. There is also a substantial burden on the cells during the manufacturing process resulting in variable functionality of the end product. The alternative is an allogeneic (non-self) therapy which could be manufactured in large batches and readily given as an “off-the-shelf” universal product. Given the previously mentioned benefits of iPSCs having the capacity for expansion and the ability to differentiate into other cell types, including those of the immune system, the allogeneic iPSC holds special promise for a wide range of advanced therapies.

The Immune Response Problem

To realise the promise of iPSC-derived T cells for universal immune oncology therapies the allogeneic product must not only harbor the cancer-specific payload (usually an engineered T cell receptor), but also be immune-neutral to the host

to avoid debilitating graft vs. host disease (GvHD), where the iPSC-derived transplanted immune cells regard the recipient’s body as foreign and begin to attack. Being immune-neutral is also important for iPSC or iPSC-derived transplanted non-immune cells in regenerative therapies to ensure they are not simply cleared from the body by the host immune system before they can have an effect.

The immune system uses a sophisticated complex of genes, expressed on the cell surface as peptides, to recognize self from non-self. Most notably the major histocompatibility complex (MHC) class I and class II glycoproteins present peptides to the T cell receptors. In humans, MHC class I and MHC class II are encoded by the human leukocyte antigen system (HLA) and HLAII, respectively). Research in the use of iPSCs for regenerative medicine has shown that knocking out $\beta 2$ microglobulin (B2M) and class II major histocompatibility class transactivator (CIITA), which are critical for the HLA/II display on the cellular surface, can render iPSC-derived cells less susceptible to immune rejection in an allogeneic therapy.⁵ To further improve compatibility iPSCs can be engineered to express HLA-E as their only surface HLA class I molecule, which prevents NK cell lysis of B2M $-/-$ cells.⁶

Allogeneic primary T cells can be engineered to avoid GvHD by removal of the T cell receptor α (TCR) and CD52 genes.⁷ Further engineering to knock out B2M and programmed cell death protein 1 (PDCD1) can additionally enhance T cell therapy function.⁸ Still, the expansion protocols required for editing primary T cells can also result in exhausted cells and less effective products. Although NK cells naturally perform their function regardless of the patient’s HLA haplotype, they have a limited proliferative capacity that makes genetic engineering of this cell type *ex vivo* extremely challenging. Because both T and NK cells can be derived from iPSCs, the logic holds that iPSCs edited to be allogeneic medicines could serve as a better universal starting point for these oncology treatments.

CRISPR Revolution

With the discovery and adoption of the

robust CRISPR-Cas9 gene editing system, knockout and knock-in capabilities are well understood and have brought the promise of allogeneic products within reach, though this method of gene editing is not without limitations. Type II nucleases like Cas9 introduce knockout through a targeted double strand DNA (dsDNA) break in the genome. When the cell uses inherent DNA repair pathways to repair the damage the dsDNA is often repaired incorrectly. The resulting insertions and deletions (INDELS) cause the target protein to be non-functional or not transcribed at all, which knocks out the protein function. Following the same process, the targeted dsDNA break can be supplemented with an exogenously delivered DNA repair template, which can incorporate new sequence into the broken locus through endogenous homology directed repair mechanisms and thereby knock in new protein function. Sequence-specific targeting limitations and off-target INDELS aside, the dsDNA break induced by Cas9 is a catastrophic and often fatal event for the cell, particularly for the sensitive iPSCs. Furthermore, when multiple dsDNA breaks are introduced in the same cell at the same time (whether it's two on-target genes in a multiplex knockout experiment or one on-target and one off-target gene in a singleplex knockout experiment) the cut ends can translocate, resulting in large genomic rearrangements that can lead to oncogenic phenotypes.⁹

One way to get around this potentially fatal flaw draws on the iPSC's ability to persist through many divisions in culture. Gene editing with CRISPR-Cas9 is done sequentially and at each round, clonal cell lines are isolated, grown, and analysed for precise editing, genomic integrity, and stem cell health. This intensive effort plays out over weeks to months and is in theory undertaken four times in the case of the sequential B2M/CD52/TRAC/PDCD1 knockouts required to create the allogeneic iPSC-derived T cell product. While the iPSCs are capable of significant expansion in culture, each round of division (roughly every 24 hours) may have additive negative impact on the health of the cell culture where spontaneous karyotypical abnormalities and loss of pluripotency tend to arise over time, even in clonal cell lines.

Base Editing: Enhancing iPSC Engineering

To speed up and de-risk the generation of allogeneic iPSC products one could consider

next-generation gene editing approaches which do not introduce dsDNA breaks, such as base editing. Avoidance of dsDNA breaks is an improved safety feature that enables multiplex editing in a single step. Compared to CRISPR-Cas9 knockout which relies on INDEL formation, base editing introduces nucleotide-specific changes to the DNA and can cause a knockout phenotype through the introduction of a premature stop codon or a splice site disruption.^{10,11}

Base editing (Figure 1) also utilises an RNA-guided nuclease like Cas9, but the nuclease is modified in one of two ways: (i) The DNA cleavage domains are either fully deactivated (dCas9) or (ii) one domain is deactivated to create a nuclease that can nick but not cut DNA (nickase, nCas9). While the deactivated enzyme is considered to have less unintended effects, the nickase gives increased efficiency.¹² The deamination of the target base happens only on one strand, so by nicking the single DNA strand opposite to the edited base the cell is

forced to repair the DNA using the edited strand as the template, thus improving the likelihood of the edit being incorporated in the next round of DNA replication. Utilising base editing for multiplexed knockout is particularly advantageous as there is less damage to the genome and less chance for genomic translocations between edited loci when no dsDNA breaks occur.¹³ This results in healthier cells in a shorter amount of time at reduced cost.

Beyond its application in the creation of next-generation adoptive T cell therapies, it is expected that base editing will offer similar opportunities for the engineering of a wide range of allogeneic cell therapies with increasingly advanced safety and functionality profiles. The continued development of advanced base editors will set the stage for affordable off-the-shelf allogeneic treatments for a broad range of indications. With advances in gene editing technologies, the iPSC may have found its place in allogeneic therapies.

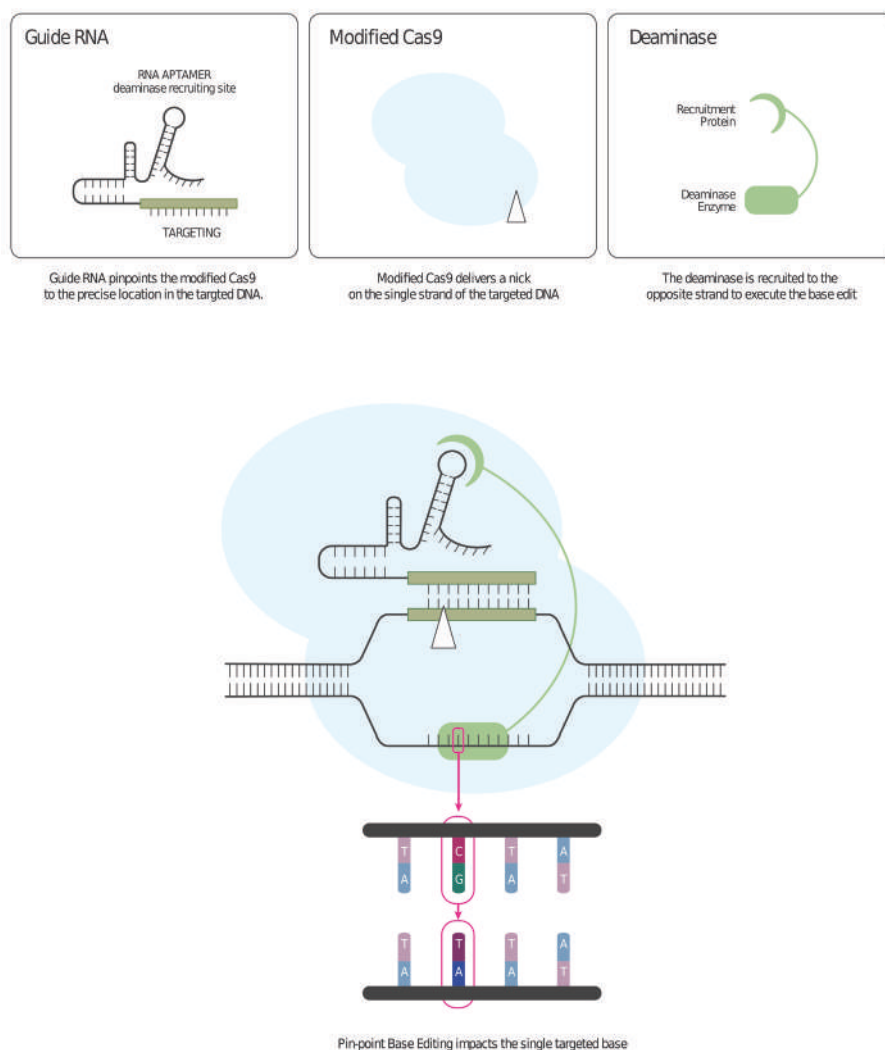
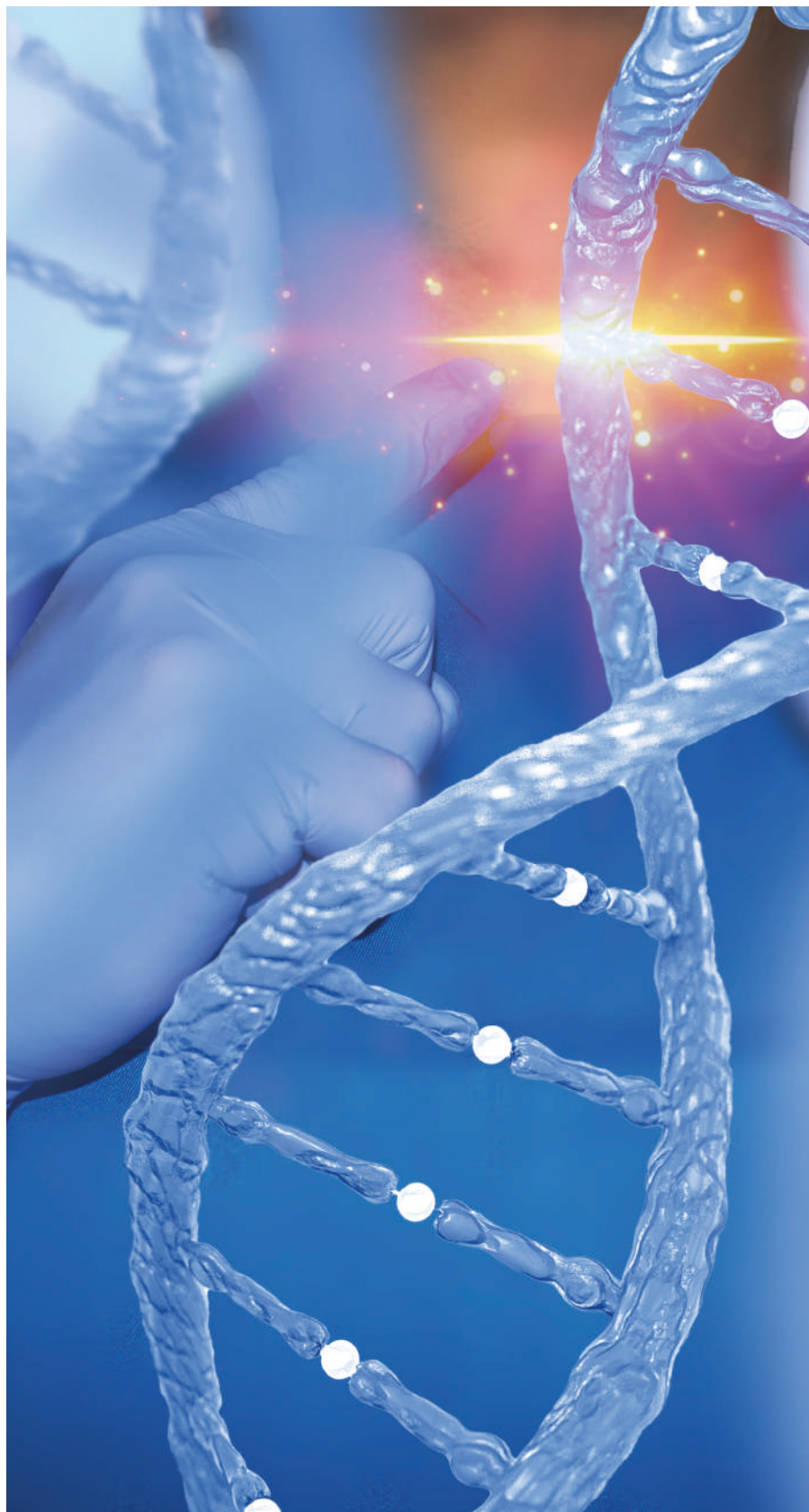


Figure 1: Base editing technology utilising a nickase form of Cas9 to elicit precise single strand cuts at target sites in the genome. Illustration by Revvity, inc.

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