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A knockout run for CRISPRed cells

The continuing optimization of the safety and applicability of CRISPR for ex vivo genome editing will broaden the prospects of cell therapies.

The first regulatory approval of a treatment using a particular technology or mode of action is typically consequential. Even when other drugs or therapies for the same medical need are available, such 'first-in-modality' approvals are a recognition of the scientific and technology development efforts that came before, and can be a boon for patients, the involved biotechnology or pharmaceutical companies, and the related scientific, business and social ecosystems. This was the case for rituximab, the first monoclonal antibody for the treatment of cancer, approved in 1997 by the US Food and Drug Administration (FDA) initially for the treatment of non-Hodgkin's lymphoma; and for Luxturna (voretigene neparvovec) in 2017, the first FDA-approved gene therapy using an adeno-associated virus as a vector, for the treatment of the inherited retinal disease Leber congenital amaurosis (Luxturna delivers a functional copy of the *RPE65* gene to retinal cells). These treatments led to frenzied research and development in targeted cancer drugs and in in vivo gene therapies for the central nervous system. Will a treatment using CRISPR (clustered regularly interspaced short palindromic repeats) for the ex vivo editing of therapeutic cells soon follow?

It is widely expected that the FDA will give the green light to using autologous $CD34^+$ human haematopoietic stem cells and progenitor cells edited ex vivo via CRISPR–Cas9 for the treatment of the haemoglobinopathies sickle cell disease and β-thalassaemia[1]. The cell product, developed by Vertex and CRISPR Therapeutics, leverages electroporated Cas9 to knock down *BCL11A*, a gene repressing the production of foetal haemoglobin, in the haematopoietic cells harvested from the patient, and may provide superior longer-term benefits than current pharmacological therapies, which only treat the disease's symptoms.

Many more CRISPR-based cell therapies for haemoglobinopathies and other monogenic diseases with considerable medical need, particularly cancers, primary immunodeficiencies and inherited metabolic disorders, are being tested in clinical trials or are in preclinical development[2]. This issue of *Nature Biomedical Engineering* includes a latest example: in an Article, David Liu and colleagues show that prime editing can efficiently correct the sickle-cell allele to produce wild-type haemoglobin in human haematopoietic stem cells that then engraft efficiently in mice, yielding erythrocytes that are resistant to hypoxia-induced sickling. Prime editing is a newer CRISPR modality, first reported by Liu and collaborators in 2019 (ref. 3), that uses a guide RNA designed to specify the target locus and to encode the desired edit alongside a deactivated Cas9 nicking enzyme fused to a reverse transcriptase (depicted), to copy the desired edit into the target locus. As highlighted by Sébastien Levesque and Daniel Bauer in the accompanying News & Views article, "a central advantage of therapeutic prime editing is the genome-wide safety profile of the technique and its high level of product purity."

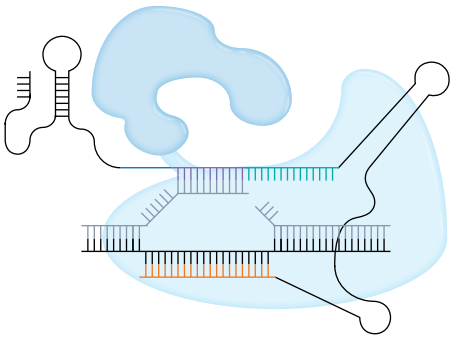

Figure adapted with permission from the News & Views article by Levesque and Bauer.

Beyond safety factors (which largely relate to off-target edits and to the immunogenicity of the edited cells[4]), there are considerations of efficacy, durability and manufacturing that are being worked on to improve the translatability of CRISPR technology for the ex vivo editing of therapeutic cells. These include the optimization of the delivery and expression of the CRISPR payload, the enhancement of the long-term phenotypic stability of the edited cells, and the development of cost-effective methods for their manufacturing and quality control[5,6]. Three more Articles included in this issue exemplify such efforts.

Manufacturing autologous and allogeneic engineered T cells for cell therapy has largely relied on electroporation, which is cytotoxic to cells and expensive to implement. Ross Wilson and colleagues now show a gentler, simpler, cheaper and more efficient method for the production of cell-therapy products based on primary human T cells: the use of amphiphilic peptides to aid the cellular uptake of CRISPR ribonucleoprotein (the authors used Cas9 and Cas12a nucleases as well as an adenine base editor). The peptides were identified through screening for consistently producing high yields of edited cells (as the authors show for T cells, B cells and natural killer cells). In the accompanying News & Views article, Julian Grünewald and Andrea Schmidts note that the method's minimal impact on gene expression and on functional phenotypes may also facilitate the delivery of double knock-ins into T cells at efficiencies that are comparable to electroporation yet with the benefit of reduced cytotoxicity. An analogous method, recently published in *Nature Biotechnology*, shows the efficiency and low cellular toxicity of the delivery of Cas9 and Cas12 nucleases fused with cell-penetrating peptides into T cells and haematopoietic progenitor cells when assisted by fusion peptides aiding cell penetration and endosomal escape[7].

Retroviruses can be used to engineer primary human cells to stably express a large transgene. However, transgene expression is often lost, owing to defensive transcriptional silencing of the inserted sequences. This can be avoided by leveraging homologous directed repair and adeno-associated viruses to knock-in the transgene into essential endogenous genomic loci. However, this strategy limits the size of the payload that can be delivered and is more toxic to cells. Lei Qi and co-authors now report a method for the knock-in and stable expression of large payloads: it uses an integrase-deficient lentivirus to insert the encoded payload (flanked by 'homology arms' and 'cut sites') upstream of an endogenous essential gene, followed by the delivery of the CRISPR ribonucleoprotein via electroporation.

Editorial

To improve the safety of CRISPR-edited cell therapies, controlled inhibition of the activity of Cas9 and other RNA-guided nucleases is arguably the most straightforward and powerful strategy. Masaki Kawamata, Hiroshi Suzuki, Atsushi Suzuki and colleagues now show an alternative approach: the inhibition of the formation of functional Cas9 ribonucleoproteins via the addition of cytosine stretches to the 5′-end of single-guide RNAs. In human pluripotent stem cells, short cytosine extensions reduced enhanced homology-directed repair while maintaining bi-allelic editing, whereas longer cytosine extensions decreased on-target activity yet improved the specificity and precision of mono-allelic editing. The authors found optimal extensions that led to windows of Cas9 activity associated with low cytotoxicity and with negligible activation of the DNA-damage-sensor protein p53.

Cell therapies and T cell immunotherapies for treating haemoglobinopathies, B cell cancers and primary immunodeficiencies require toxic myeloablative chemotherapy before transplantation of the cells, to kill the defective stem cells and progenitor cells or the malignant cells in the bone marrow and to facilitate the engraftment of the therapeutic cells. Yet all the technological innovations highlighted here could, in principle, be further developed to make it possible to safely engineer the cells in vivo. That would be an utter knockout.

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