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Editorial

Emerging therapeutic applications of CRISPR genome editing

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The rapid evolution of tools for genome editing has created a dizzying array of possibilities for novel therapeutic strategies, even though to date only a handful of clinical applications have been realised. Proof-of-concept demonstrations of targeted genome modification *in vitro* and in small animal models of inherited single gene disorders have to be translated into effective therapies. Interest has naturally gravitated towards opportunities for collection, *ex vivo* modification and return of blood, immune and stem cells. Initial applications designed to modify T cells to protect against HIV or to confer potent anti-leukaemic effects have reached clinical phase, and further applications to modify blood stem cells are close to being applied. There are generic considerations of safety, on- and off-target effects and possible genotoxicity as well as issues relating to more sophisticated systemic approaches where niche occupation and host immunity become relevant. Such issues will be likely addressed over time, with carefully designed clinical trials required to determine therapeutic risks and benefits.

First-in-human applications of genome editing are being realised, albeit in small numbers of subjects. Initially, zinc finger nuclease (ZFN) technology was shown to efficiently disrupt expression of the HIV co-receptor, chemokine receptor 5 (CCR5), in T cells. This *ex vivo* strategy was employed to confer resistance to HIV and provided proof-of-concept data that cell editing and reinfusion were both feasible and safe [1]. While the studies in HIV did not demonstrate efficacy, the technical success of being able to modify human T cells, and later haematopoietic stem cells (HSC), on a clinical scale and in a compliant manner was an important milestone. Subsequently, in 2015, the combined application of lentiviral transduction and transcription activator-like effector nucleases (TALENs) to generate 'universal' non-HLA-matched anti-leukaemic T cells provided early evidence of the life-saving potential of genome-edited cells [2]. The universal T-cell strategy is currently being evaluated in ongoing multicentre studies, and similar approaches have employed other platforms including ZFNs [3], Meganucleases [4] and CRISPR/Cas9 [5]. The first report of a therapeutic CRISPR application emerged in 2016, when researchers from Chengdu in China were reported to have treated a patient with cells modified using CRISPR/Cas9 to disrupt PD-1, a checkpoint inhibitor pathway, in the hope of enhancing lymphocyte responses in lung cancer [6]. The outcomes are as yet unpublished but many similar studies were proposed for a variety of other malignancies. Then, most recently, in early 2019, *CRISPR Therapeutics* announced that a first patient has been treated using genome-edited autologous HSCs, engineered to deliver increased foetal haemoglobin in red cells in subjects with β-thalassaemia. This special issue of *Emerging Topics in Life Sciences* assembles state-of-the-art updates related to translational applications of CRISPR technology relevant to human therapies. The focus is on the modification of somatic tissues for human health, where the ethical debate is generally supportive and less contentious especially in relation to serious, often life-threatening, diseases. Applications of genome modification of the germline and embryonic genome editing are not considered here, other than to note current international calls for a formalised moratorium on such procedures [7].

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Whereas existing gene therapy approaches largely rely on near-random insertion of a transgene under the control of a constitutive promoter, the targeted gene insertion mediated by genome editing offers the possibility of highly specific gene correction with physiologically regulated gene expression. In the case of monogenic disorders with recessive loss-of-function mutations (e.g. cystic fibrosis), targeted insertion of a 'repair template' by homologous recombination would ideally allow correction of a specific mutation or at least insertion of cDNA transgene under the control of a native promoter. In other cases, dominant negative effects might be suppressed through allele-specific inactivation effects of non-homologous end joining (NHEJ).

The clustering of so many initial applications of genome modification for blood and immune disorders reflects the relative ease and established infrastructure for undertaking collection, manipulation and return of lymphocytes and bone marrow-derived stem cells. T cells have been manipulated and expanded in many gene therapy trials without notable toxicity and there have been no reports of transformation in such studies. Preece and Georgiadis [8] review the potential of genome-edited T cells, focussing on chimeric antigen receptor (CAR)-modified cells for the treatment of blood malignancies, and advantages of genome editing of the T-cell receptor, HLA molecules or other critical molecules important for immune function. Similar *ex vivo* approaches targeting HSCs are more challenging, given the risks of genotoxicity uncovered in the past retroviral gene therapy trials, and the predisposition to activation of apoptotic pathways following manipulations, in particular after double-stranded DNA breakage [9,10]. Strategies to manipulate these pathways may be required for efficient, high yield editing of stem cell populations. Romito et al. [11] provide an update on the potential of genome editing of blood stem cells, and DeRavin and Brault [12] consider specific applications for inherited immunodeficiencies, some of which are close to clinical phase testing. The attraction of such conditions for genome editing derives from the possibility of providing therapeutic 'cure' through the correction of a relatively modest number of stem cells if upon correction, cells acquire a powerful survival advantage compared with defective populations. The same rationale was originally applied in relation to 'gene-addition' using viral vectors for the correction of autologous HSC defects. In some cases, these approaches now offer an alternative to bone marrow transplantation if suitable donors are not available. The inclusion of highly precise genome modification offered by CRISPR/Cas9 and other editing platforms seem likely to be adopted for improved versions of existing treatments, or as alternatives with improved risk: benefit profiles. More broadly, Lawrence et al. [13] consider the application of genome editing to create a supply of 'universal' blood products such as platelets, which if successful, could eventually address matching and supply issues across healthcare.

Direct *in vivo* applications are also being evaluated, including injection of adenoviral-associated vectors (AAV) encoding ZFNs for targeted insertion of transgenes to treat inherited mucopolysaccharidoses. The subjects treated to date are limited, and detailed follow-up is awaited to determine if sufficient modification was achieved, but the trials represent important early milestones for direct in human therapies. The challenges of delivering systemic therapy are notable, but for certain conditions will have to be systematically addressed. The issue is highlighted by inherited skin conditions such as epidermolysis bullosa, where successful grafting of vector-corrected autologous epidermal sheets had been reported, but direct *in vivo* approaches are required for systemic benefit with targeted delivery to the skin and mucosa. A review of the status of genome editing for skin conditions by Naso and Petrova [14] provides insights into the wider application of the technology. The hurdles of delivering guides, nuclease and homology flanked repair sequences may require combinations of viral and non-viral technologies to achieve efficient correction in a variety of conditions. Additional aspects relevant to direct *in vivo* therapies include the likely immunogenicity of bacterial-derived Cas proteins. Antibody and T-cell responses against *Streptococcus* or *Staphylococcus*-derived Cas9 have been reported in over half of healthy individuals [15]. Possible intracellular sensing and innate responses against CRISPR guides may also be problematic, although can be abrogated through chemical modifications such as 2′-O-methylation [16]. In addition, possible immune responses against viral components from delivery vectors may be encountered following *in vivo* applications.

Characterisation of modified cells through multi-modal examinations may include at the molecular level targeted quantification of genomic signatures of on- and off-target effects using quantitative or digital PCR and high-throughput sequencing if the target sites are well defined. Targeted screening for predicted translocations may be possible, or unbiased screening may be included using FISH and karyotype analysis. Such approaches are considered by Gkazi [17], and the relevant advantages and limitations discussed. This rapidly evolving area is being fashioned by technological improvements including sequencing accuracy, depth of reads and enhanced bioinformatics. Yet, the predictive value of such information in terms of risks of genotoxicity or possible adverse effects is nebulous, and it seems likely that only clinical experience with careful mapping of possible

toxicities will define actual risks of particular editing interventions. Here, the experience following the first trials using γ-retroviral vectors to treat childhood immunodeficiencies, such as severe combined immunodeficiency [18], chronic granulomatous disease [19] and Wiskott–Aldrich syndrome [20], is informative. Unanticipated vector-mediated insertional mutagenesis manifested in patients many months or years after treatment, and in subsequent analyses, certain vector insertion sites were considered high risk, but the predictive value of integration site mapping in determining transformation risk remains limited. Similarly, self-inactivating lentiviral vector profiles were considered less likely to mediate insertional transactivation but could nonetheless disrupt transcriptional regulation and cause clonal expansion [21]. Thus, while genome editing effects should be carefully mapped and documented in engineered products, the full significance of any modifications will likely only be uncovered in human studies and may require extended monitoring for many years to be fully appreciated.

Finally, the genome editing toolbox continues to evolve and expand, with a myriad of possibilities and applications for human therapies. Generic issues in relation to delivery strategies, molecular mapping and patient monitoring regimens will remain, but it seems likely that ever efficient, precise and versatile editing will become accessible for a broad range of applications.

Abbreviations

AAV, adenoviral-associated vectors; CAR, chimeric antigen receptor; CCR5, chemokine receptor 5; HSC, haematopoietic stem cells; NHEJ, non-homologous end joining; TALENs, transcription activator-like effector nucleases; ZFN, zinc finger nuclease.

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Competing Interests

W.Q. holds equity in Autolus and Orchard Therapeutics.

References

1 Tebas, P., Stein, D., Tang, W.W., Frank, I., Wang, S.Q., Lee, G. et al. (2014) Gene editing of CCR5 in autologous CD4T cells of persons infected with HIV. *N. Engl. J. Med.* **370**, 901–910 https://doi.org/10.1056/NEJMoa1300662
2 Qasim, W., Zhan, H., Samarasinghe, S., Adams, S., Amrolia, P., Stafford, S. et al. (2017) Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells. *Sci. Transl. Med.* **9**, eaaj2013 https://doi.org/10.1126/scitranslmed.aaj2013
3 Cooper, M.L., Choi, J., Staser, K., Ritchey, J.K., Devenport, J.M., Eckardt, K. et al. (2018) An "off-the-shelf" fratricide-resistant CAR-T for the treatment of T cell hematologic malignancies. *Leukemia* **32**, 1970–1983 https://doi.org/10.1038/s41375-018-0065-5
4 MacLeod, D.T., Antony, J., Martin, A.J., Moser, R.J., Hekele, A., Wetzel, K.J. et al. (2017) Integration of a CD19 CAR into the TCR alpha chain locus streamlines production of allogeneic gene-edited CAR T cells. *Mol. Ther.* **25**, 949–961 https://doi.org/10.1016/j.ymthe.2017.02.005
5 Eyquem, J., Mansilla-Soto, J., Giavridis, T., van der Stegen, S.J., Hamieh, M., Cunanan, K.M. et al. (2017) Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. *Nature* **543**, 113–117 https://doi.org/10.1038/nature21405
6 Cyranoski, D. (2016) Chinese scientists to pioneer first human CRISPR trial. *Nature* **535**, 476–477 https://doi.org/10.1038/nature.2016.20302
7 Lander, E.S., Baylis, F., Zhang, F., Charpentier, E., Berg, P., Bourgain, C. et al. (2019) Adopt a moratorium on heritable genome editing. *Nature* **567**, 165–168 https://doi.org/10.1038/d41586-019-00726-5
8 Preece, R. and Georgiadis, C. (2019) Emerging CRISPR/Cas9 applications for T-cell gene editing. *Emerging Topics in Life Sciences* **3**(3), https://doi.org/10.1042/ETLS20180144
9 Ihry, R.J., Worringer, K.A., Salick, M.R., Frias, E., Ho, D., Theriault, K. et al. (2018) P53 inhibits CRISPR-Cas9 engineering in human pluripotent stem cells. *Nat. Med.* **24**, 939–946 https://doi.org/10.1038/s41591-018-0050-6
10 Haapaniemi, E., Botla, S., Persson, J., Schmierer, B. and Taipale, J. (2018) CRISPR-Cas9 genome editing induces a p53-mediated DNA damage response. *Nat. Med.* **24**, 927–930 https://doi.org/10.1038/s41591-018-0049-z
11 Romito, M., Rai, R., Thrasher, A.J. and Cavazza, A. (2019) Genome editing for blood disorders: state of the art and recent advances. *Emerging Topics in Life Sciences* **3**(3), https://doi.org/10.1042/ETLS20180147
12 DeRavin, S.S. and Brault, J. (2019) CRISPR/Cas9 applications in gene therapy for primary immunodeficiency diseases. *Emerging Topics in Life Sciences* **3**(3), https://doi.org/10.1042/ETLS20180157
13 Lawrence, M., Mueller, A. and Ghevaert, C. (2019) Using genome editing to engineer universal platelets. *Emerging Topics in Life Sciences* **3**(3), https://doi.org/10.1042/ETLS20180153
14 Naso, G. and Petrova, A. (2019) CRISPR/Cas9 gene editing for genodermatoses: progress and perspectives. *Emerging Topics in Life Sciences* **3**(3), https://doi.org/10.1042/ETLS20180148
15 Charlesworth, C.T., Deshpande, P.S., Dever, D.P., Camarena, J., Lemgart, V.T., Cromer, M.K. et al. (2019) Identification of preexisting adaptive immunity to Cas9 proteins in humans. *Nat. Med.* **25**, 249–254 https://doi.org/10.1038/s41591-018-0326-x
16 Yin, H., Song, C.Q., Suresh, S., Wu, Q., Walsh, S., Rhym, L.H. et al. (2017) Structure-guided chemical modification of guide RNA enables potent non-viral in vivo genome editing. *Nat. Biotechnol.* **35**, 1179–1187 https://doi.org/10.1038/nbt.4005

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https://doi.org/10.1042/ETLS20190010

17 Gkazi, S.A. (2019) Quantifying CRISPR off-target effects. *Emerging Topics in Life Sciences* **3**(3), https://doi.org/10.1042/ETLS20180146
18 Hacein-Bey-Abina, S., Hauer, J., Lim, A., Picard, C., Wang, G.P., Berry, C.C. et al. (2010) Efficacy of gene therapy for X-linked severe combined immunodeficiency. *N. Engl. J. Med.* **363**, 355–364 https://doi.org/10.1056/NEJMoa1000164
19 Ott, M.G., Schmidt, M., Schwarzwaelder, K., Stein, S., Siler, U., Koehl, U. et al. (2006) Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EVI1, PRDM16 or SETBP1. *Nat. Med.* **12**, 401–419 https://doi.org/10.1038/nm1393
20 Boztug, K., Schmidt, M., Schwarzer, A., Banerjee, P.P., Diez, I.A., Dewey, R.A. et al. (2010) Stem-cell gene therapy for the Wiskott-Aldrich syndrome. *N. Engl. J. Med.* **363**, 1918–1927 https://doi.org/10.1056/NEJMoa1003548
21 Cavazzana-Calvo, M., Payen, E., Negre, O., Wang, G., Hehir, K., Fusil, F. et al. (2010) Transfusion independence and HMGA2 activation after gene therapy of human β-thalassaemia. *Nature* **467**, 318–322 https://doi.org/10.1038/nature09328