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ARTICLE COMMENTARY

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The future of ex vivo hematopoietic stem cell gene editing: what's next

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Monogenic disorders have been a focus of research for a long time, with scientists endeavoring to develop gene therapy strategies aimed at correcting, replacing, or adding therapeutic genes to the cells of interest. Blood disorders have pioneered this effort. One of the reasons is that the precursors of all blood cells, hematopoietic stem and progenitor cells (HSPCs), are easily accessible and can be manipulated outside the body before being reinfused into the patient. The procedure resembles HSPC transplantation, but as it uses patient's own stem cells, there is no risk of rejection or graft versus host disease, resulting in relatively milder preparative conditioning regimens. The first *ex vivo* gene therapy trials used gamma retroviral vectors to add genes of interest to HSPCs, leading to highs and lows, the latter mainly caused by insertional mutagenesis and/or loss of correction over time. Since then, gene addition strategies have been refined further and nowadays self-inactivating lentiviral vectors are in the clinic for several hematopoietic and metabolic disorders that can be ameliorated by cross correction from blood-derived cells. Although lentiviral vectors performed well in several trials and are significantly safer than first-generation gamma retroviral vectors, they can still pose risks related to semi-random integration into the genome and nonphysiological expression patterns, particularly when heterologous or truncated versions of endogenous promoters are employed [1].

In this commentary, we will describe how gene editing has stepped in to try and solve these issues and discuss its limitations and future directions.

2. Ex vivo HSPC gene editing: the present

The relatively recent discoveries of DNA endonuclease enzymes, most notably those associated with the clustered regularly interspaced short palindromic repeats (CRISPR) system, have paved the way to seamless genetic correction in HSPCs while maintaining physiological gene regulation, standing out from viral-based gene therapy strategies [2]. CRISPR/Cas9 targets specific locations in the genome using a guide RNA–DNA pairing. Upon recognition of a cutting motif called protospacer adjacent motif (PAM), it generates a double-strand DNA break (DSB), which can be corrected by cellular DNA repair pathways, such as non-homologous end joining (NHEJ) and microhomology-mediated end joining (MMEJ). This approach has been successfully used to perform targeted disruption of regulatory regions or genes via NHEJ/MMEJ repair, as exemplified by the recent approval of Casgevy® for the treatment of β-hemoglobinopathies, where induced insertions and deletions (indels) in the erythroid enhancer of the *BCL11a* gene activate γ-globin, compensating for the loss of functional β-globin [3]. On the other hand, when a therapeutic donor sequence, flanked by homology regions encompassing the DSB, is provided alongside the CRISPR-machinery, the cell can use that template to insert the therapeutic sequence or correct endogenous sequences through homology-directed repair (HDR). Most monogenic disorders are loss of function caused by mutations spanning the whole genomic locus, and as such, a "universal" approach for these diseases is the insertion of the entire therapeutic coding sequence next to its own promoter for endogenous regulation. Encouraging results have been reported in preclinical studies in which HSPC gene editing was carried out by delivering Cas systems through electroporation of ribonucleoprotein (RNP) complexes and the HDR donor template by recombinant adeno-associated virus 6 vectors (AAV6) [4–6].

Despite the promise, currently several hurdles need to be overcome to achieve long-lasting correction of blood monogenic diseases using genome editing technologies, particularly in the context of HDR-mediated gene addition. Indeed, gene editing approaches have shown preferential genetic correction of the prevailing population of more committed progenitors, at the expense of rarer long-term repopulating stem cells (LT-HSCs), when manipulating cells *ex-vivo*. This is driven by multiple factors, including the inefficient delivery of the donor template, the quiescent nature of LT-HSCs, which reduces the activity of the HDR pathway, and the intrinsic cytotoxicity of the procedure that may limit the frequency of LT-HSCs in the infused product or their engraftment and self-renewing ability post transplantation. Scientists have welcomed the advent of NHEJ inhibitors to boost

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HDR in primitive cells, although concerns about their safety remain [7]. Reduction in the hematopoietic repopulation capacity of *ex vivo* manipulated HSPCs has been observed in numerous preclinical studies mainly caused by a p53-mediated DNA damage response to the Cas9 endonuclease activity [8–10]. One clinical trial (NCT04819841) employing CRISPR/Cas9 and an AAV6-based HDR template to deliver a working copy of the β-globin gene for the treatment of Sickle Cell Disease (SCD) demonstrated strong preclinical efficacy [11], but unsuccessful clinical outcome, with the treated patient experiencing incomplete hematopoietic reconstitution, likely caused by editing-related cell toxicity. Careful HSPC culture optimizations have been carried out to ensure maximal targeting of HSCs while preserving their phenotypic and functional properties *in vitro*. This includes the use of small molecules in cell cultures to maintain the long-term multilineage repopulation capacity of human-corrected HSPCs [9,12] or to dampen innate immune and DNA damage response [9,13], exacerbated by the editing procedure and AAV transduction. Employing low AAV6 titers or non-integrative lentiviral vectors (IDLV) could be helpful to reduce HSC differentiation and exhaustion after the gene editing procedure, but increased viral production costs, lower reproducibility, and genomic perturbations due to vector insertion or residual integrations demotivate their use [14]. Single strand DNAs as donor templates are promising candidates given the reduced toxicity observed in T cells and HSPCs [15] when compared to double strand DNA; however, knock-in frequencies are still lower than those achieved with their viral counterpart and inconsistent results have been obtained among different groups. Numerous studies have demonstrated that CRISPR systems can be delivered in primary cells as proteins by cell-penetrating peptides [16,17] or in the form of plasmid DNA, mRNA, and RNP *via* lipid nanoparticles (LNPs) [18]. When used to edit HSPCs, LNP-mediated editing reduces p53 pathway activation, promoting greater clonogenic activity and achieving similar or even better long-term repopulation capabilities compared to electroporation at similar rates of editing [19]. While promising, efficient protocols to encapsulate and deliver template DNAs via LNPs to HSCs are yet to be optimized, leaving HDR-based systems overall behind other editing approaches in the clinical trial arena.

The advent of second-generation Cas9 editors, lacking DNA double strand activity, has opened the possibility of inducing small genomic changes or correcting single mutations *in situ*, preserving regulatory sequences and introns while avoiding DSBs [2]. Base and prime editing are recent advances in this direction, pioneered by David Liu. Base editors chemically modify certain DNA bases, by fusing a Cas nickase to a cytosine (C:T conversion) or adenine (A:G conversion) deaminase. Prime editing is a technology that merges the Cas9 nickase with a reverse transcriptase (RT) and uses a long guide RNA (peg RNA) as a template to enable the creation or correction of any type of mutation, including insertions and deletions. Efforts to apply these technologies to HSPCs have initially concentrated on diseases where a single approach would benefit many patients, which have been rapidly integrated into clinical trials. Indeed, as of today five and one trials feature base and prime editors, respectively, despite these technologies being relatively recent discoveries (www.clinicaltrials.gov). The approaches have been applied to both SCD and transfusion dependent thalassemia, as exemplified by the recent clinical trial led by Beam (NCT05456880), targeting the promoter of γ-globin via base editors to induce fetal hemoglobin. Clinical trials aiming at treating X-linked (NCT06325709) and p47-deficient chronic granulomatous disease (NCT06559176) are two further examples of monogenic diseases caused by single mutations for which base and prime editing could represent a one-time, definitive treatment.

3. The (bumpy) road to clinic

Irrespective of the tool and the approach used, translation of gene editing platforms from bench to bedside requires thorough safety assessment. Genetic changes at undesired locations, RNA editing, and chromosomal aberrations involving on-and off-target sites are bystander effects that, even if dramatically reduced using high-fidelity systems, pose a significant risk [20,21]. Therefore, comprehensive preassessment of unwanted modifications and their continuous monitoring post-treatment in transplanted patients is crucial. In response to a call for a tight scrutiny of the guide RNA specificity, there has been a surge in technologies designed to assess the safety profile of each gene editor, using both quantitative and qualitative assays *in vitro* and *in cellula*. Nevertheless, the field has yet to establish standardized approaches to determine the genotoxic potential of each gene editing technology and there remains a lack of consensus regarding sample selection, methodologies, and risk thresholds for the safe release to the market of gene editing-based advanced therapy medicinal products (ATMPs). The landscape of nuclease specificity analysis is further deranged by the uncertainty over the influence of the sole cell culture procedure on genotoxicity [22] and by the lack of methods to predict the functional impact of such genomic aberrations on cells, risking overloading scientists with information that cannot be easily deciphered. Thus, another crucial milestone will be the development of functional readouts of safety specific to the therapeutic cell type of interest, which could possibly be performed *in vitro* and universally utilized, similarly to the *In Vitro* Insertional Mutagenesis assays commonly used for the release of *ex vivo* viral-based gene therapies [23]. Reproducing *in vitro* normal and abnormal hematopoiesis is particularly challenging. However, we are optimistic that with new *in vitro* 3D bone marrow reconstruction methodologies, we will soon be able to refine the study of HSPC functionality and thus facilitate the reproducible and fast detection of true genotoxic events before reaching the patient.

A major issue further complicating the road to clinical routine for HSPC gene editing is patient access to ATMPs, which involves significant conflicts between ethical and financial considerations. Cost and scalability of *ex vivo* gene editing therapies remain significant barriers to patient access, influenced by factors such as disease prevalence, regulatory requirements, and the capacity of manufacturing sites. Sharing and harmonizing standard operating procedures (SOPs) in gene editing manufacturing, along with their contribution to streamlining regulatory processes, would facilitate the development of a universal investigational new drug (IND) application, making a significant step forward in improving patient access to these therapies.

Furthermore, advancing toward closed, automated manufacturing protocols to reduce production costs and ensure greater consistency across products need systematic promotion. Post-approval strategies, such as innovative payment models, risk-sharing agreements, equity-based tiered pricing, and shared Intellectual Property/patent pooling, must also be taken into consideration to lower overall costs and increase accessibility to therapies. Regional disparities to patient access could also be met with complementary solutions of international centers of excellence for critical mass treatments and expertise on one hand – especially for rare and ultra-rare diseases –, and decentralized point-of-care manufacturing for access to treatment also in peripheral or disadvantaged regions of the world on the other hand. Initiatives such as BioCanRx in Canada and the partnership between Caring Cross and Fiocruz in Brazil are practical examples of efforts toward changing the landscape of patient accessibility to ATMPs.

4. HSPC gene editing: the future

Latest innovations in the field of gene editing for hematopoietic disorders have been driven by the urge to address its clinical translatability. As previously discussed, *ex vivo* approaches require sophisticated infrastructure and patient-specific manipulation, making them a challenge for widespread use. One way to overcome these hurdles would be to mass produce an engineered HSPC population for use in off-the-shelf therapies for many patients. For example, gene editing could be used to create hypoimmune HSPCs by knocking out components of the MHC class I molecules to evade the immune system and by overexpressing CD47, as demonstrated in induced pluripotent stem cells [24]. Effective protocols to amplify *in vitro* HSPCs or to systematically derive them from induced pluripotent stem cells are, however, still under development.

By leveraging tools such as base and prime editors, able to make precise changes with supposedly less genotoxic effects compared to DSB-inducing editors, an individual, patient-tailored and personalized approach using a regulatory approved flexible platform would also be a potential alternative to streamline regulatory approval, reduce costs, and simplify gene editing approaches. This could be envisaged as a unique, approved GMP platform that allows transitioning from correction of one mutation to another by changing only one component – for example, a "universal" LNP-delivered base editing platform that can serve any disease mutation by simply exchanging the guide RNA [25]. Technology is advancing rapidly, and with the help of AI-driven algorithms, clinicians and researchers will be able to tackle any type of mutation, reaching the goal of a personalized approach. By grouping personalized medicines per disease or even family of diseases, reduction in time and costs of testing the new platform in IND-enabling studies would be reachable. Rare and ultra-rare blood disorders are ideal models to validate this strategy, as they would mostly benefit from both a lean "preclinical testing to market approval" process, given the high morbidity and early mortality affecting the patients, and cost-accessible treatments, given the nonprofitable nature of these diseases. While we agree that many of the required preclinical experiments are informative and necessary to assess safety and efficacy, it is imperative to find a compromise between overcomplicated and lengthy approval procedures and access to therapies for those in urgent need. There is still some skepticism surrounding this model, but we are confident that this will dissolve once consensus is reached on *in vitro* potency assays and on fast, reproducible, detection of true genotoxic events for each guide RNA. New platforms using iterations of prime editing (twin editing for example) to replace DNA loci with regions of interest of varying lengths could eventually be used to substitute single exons in a gene [26]. This approach represents a middle ground between universal strategies, such as HDR-mediated gene insertion and highly personalized ones, and once optimized, it could benefit from a smooth clinical translation.

We should also consider that, in an *ex vivo* setting, the use of a myeloablative conditioning to make space in the bone marrow for a graft, even if autologous, increases the risk for the patients and the cost of care by the need for mitigating short- and long-term complications. Busulfan conditioning can lead to severe side effects like infections, bleeding, and pulmonary toxicity, leading to death in the most severe cases. Efforts have concentrated on alternative approaches, the most successful being the use of immunotoxins conjugated to a monoclonal antibody targeting a specific antigen of HSPCs. Excellent results in preclinical studies and early clinical trials motivate their use as a standalone nontoxic regimen for the gene therapy of patients with inborn errors of immunity [27,28].

A parallel way forward to avoid toxic conditioning regimens would be to mediate genetic correction of HSPCs directly into the body. Attempts to deliver editing components *in vivo* in murine HSPCs have been recently made with mixed but promising results, by delivering CRISPR components via adenoviral vectors, virus-like particles, or HSPC-targeted LNPs [18]. Being at its early stages, HSPC *in vivo* gene editing still carries some barriers that must be overcome before attaining the desired efficacy and safety standards essential for therapeutics, such as immunogenicity of CRISPR proteins and delivery vehicles, low HSPC specificity, and risk of systemic toxicity. The inefficient targeting of HSPCs *in vivo* has so far resulted in low frequency of gene correction, making the approach possible only for diseases with a strong selective advantage. One way to provide such an advantage is by using antibody-mediated conditioning while combining HSPC therapeutic editing with epitope editing of the antigen recognized by the same antibody. This strategy was recently demonstrated in a SCD setting, where CD117 antibody conditioning, coupled with multiplex base editing, successfully rescued fetal hemoglobin expression in nonhuman primates [29]. Efforts to implement *in vivo* gene therapies for HSPCs would spare the need for complex and expensive cell manipulation, toxic bone marrow conditioning, and lengthy inpatient care, enhancing simplicity and making regenerative medicine finally accessible to low- and middle-income countries.

Disclosure statement

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References

1. Duncan CN, Bledsoe JR, Grzywacz B, et al. Hematologic cancer after gene therapy for cerebral adrenoleukodystrophy. N Engl J Med. 2024 Oct 10;391(14):1287–1301.
2. Pacesa M, Pelea O, Jinek M. Past, present, and future of CRISPR genome editing technologies. Cell. 2024 Feb 29;187(5):1076–1100.
3. Frangoul H, Locatelli F, Sharma A, et al. Exagamglogene autotemcel for severe sickle cell disease. N Engl J Med. 2024 May 9;390 (18):1649–1662.
4. Meng Q, Sun H, Liu J. Precise somatic genome editing for treatment of inborn errors of immunity. Front Immunol. 2022;13:960348.
5. Rai R, Romito M, Rivers E, et al. Targeted gene correction of human hematopoietic stem cells for the treatment of Wiskott - Aldrich syndrome. Nat Commun. 2020 Aug 12;11(1):4034.
6. Bahal S, Zinicola M, Moula SE, et al. Hematopoietic stem cell gene editing rescues B-cell development in X-linked agammaglobulinemia. J Allergy Clin Immunol. 2024 Jul;154(1):195–208 e8.
7. Cullot G, Aird EJ, Schlapansky MF, et al. Genome editing with the hdr-enhancing DNA-PKcs inhibitor AZD7648 causes large-scale genomic alterations. Nat Biotechnol. 2024 Nov 27.
8. Schiroli G, Conti A, Ferrari S, et al. Precise gene editing preserves hematopoietic stem cell function following transient p53-mediated DNA damage response. Cell Stem Cell. 2019 Apr 4;24(4):551–565 e8.
9. Ferrari S, Jacob A, Beretta S, et al. Efficient gene editing of human long-term hematopoietic stem cells validated by clonal tracking. Nat Biotechnol. 2020 Nov;38(11):1298–1308.
10. Lee BC, Gin A, Wu C, et al. Impact of CRISPR/HDR editing versus lentiviral transduction on long-term engraftment and clonal dynamics of HSPCs in rhesus macaques. Cell Stem Cell. 2024 May 2;31(5):788.
11. Lattanzi A, Camarena J, Lahiri P, et al. Development of beta-globin gene correction in human hematopoietic stem cells as a potential durable treatment for sickle cell disease. Sci Transl Med. 2021 Jun 16;13(598):eabf2444.
12. Rai R, Naseem A, Vetharoy W, et al. An improved medium formulation for efficient ex vivo gene editing, expansion and engraftment of hematopoietic stem and progenitor cells. Mol Ther Methods Clin Dev. 2023 Jun 8;29:58–69.
13. Della Volpe L, Midena F, Vacca R, et al. A p38 MAPK-ROS axis fuels proliferation stress and DNA damage during CRISPR-Cas9 gene editing in hematopoietic stem and progenitor cells. Cell Rep Med. 2024 Nov 19;5(11):101823.
14. Ferrari S, Jacob A, Cesana D, et al. Choice of template delivery mitigates the genotoxic risk and adverse impact of editing in human hematopoietic stem cells. Cell Stem Cell. 2022 Oct 6;29 (10):1428–1444 e9.
15. Shy BR, Vykunta VS, Ha A, et al. High-yield genome engineering in primary cells using a hybrid ssDNA repair template and small-molecule cocktails. Nat Biotechnol. 2023 Apr;41(4):521–531.
16. Foss DV, Muldoon JJ, Nguyen DN, et al. Peptide-mediated delivery of CRISPR enzymes for the efficient editing of primary human lymphocytes. Nat Biomed Eng. 2023 May;7(5):647–660.
17. Zhang Z, Baxter AE, Ren D, et al. Efficient engineering of human and mouse primary cells using peptide-assisted genome editing. Nat Biotechnol. 2024 Feb;42(2):305–315.
18. Cavazza A, Molina-Estévez FJ, Reyes ÁP, et al. Advanced delivery systems for gene editing: a comprehensive review from the GenE-HumDi COST action working group. Mol Ther Nucleic Acids. 2025;36(1):102457.
19. Vavassori V, Ferrari S, Beretta S, et al. Lipid nanoparticles allow efficient and harmless ex vivo gene editing of human hematopoietic cells. Blood. 2023 Aug 31;142(9):812–826.
20. Turchiano G, Andrieux G, Klermund J, et al. Quantitative evaluation of chromosomal rearrangements in gene-edited human stem cells by CAST-Seq. Cell Stem Cell. 2021 Jun 3;28(6):1136–1147 e5.
21. Fiumara M, Ferrari S, Omer-Javed A, et al. Genotoxic effects of base and prime editing in human hematopoietic stem cells. Nat Biotechnol. 2024 Jun;42(6):986–986.
22. Zeng J, Nguyen MA, Liu P, et al. Gene editing without ex vivo culture evades genotoxicity in human hematopoietic stem cells. Cell Stem Cell. 2024 Dec 11;191–208.e11.
23. Modlich U, Navarro S, Zychlinski D, et al. Insertional transformation of hematopoietic cells by self-inactivating lentiviral and gammaretroviral vectors. Mol Ther. 2009 Nov;17(11):1919–1928.
24. Deuse T, Hu X, Gravina A, et al. Hypoimmunogenic derivatives of induced pluripotent stem cells evade immune rejection in fully immunocompetent allogeneic recipients. Nat Biotechnol. 2019 Mar;37(3):252–258.
25. Urnov FD. Give cas a chance: an actionable path to a platform for CRISPR cures. Crispr J. 2024 Oct;7(5):212–219.
26. Chen PJ, Liu DR. Prime editing for precise and highly versatile genome manipulation. Nat Rev Genet. 2023 Mar;24(3):161–177.
27. Uchida N, Stasula U, Demirci S, et al. Fertility-preserving myeloablative conditioning using single-dose CD117 antibody-drug conjugate in a rhesus gene therapy model. Nat Commun. 2023 Oct 12;14(1):6291.
28. Yeung J, Liao A, Shaw M, et al. Anti-CD45 pbd-based antibody-drug conjugates are effective targeted conditioning agents for gene therapy and stem cell transplant. Mol Ther. 2024 Jun 5;32 (6):1672–1686.
29. Demirci S, Mondal N, Austin W, et al. CD117 antibody conditioning and multiplex base editing enable rapid and robust fetal hemoglobin reactivation in rhesus autologous transplantation model. Blood ASH Annual Meeting Abstracts. 2024 Nov 5;144:516–517.