Targeted Gene Delivery: Where to Land

Giulia Pavani† and Mario Amendola*

INTEGRARE, UMR_S951, Genethon, Inserm, Univ Evry, Univ Paris-Saclay, Evry, France

Genome-editing technologies have the potential to correct most genetic defects involved in blood disorders. In contrast to mutation-specific editing, targeted gene insertion can correct most of the mutations affecting the same gene with a single therapeutic strategy (gene replacement) or provide novel functions to edited cells (gene addition). Targeting a selected genomic harbor can reduce insertional mutagenesis risk, while enabling the exploitation of endogenous promoters, or selected chromatin contexts, to achieve specific transgene expression levels/patterns and the modulation of disease-modifier genes. In this review, we will discuss targeted gene insertion and the advantages and limitations of different genomic harbors currently under investigation for various gene therapy applications.

Keywords: genome editing, gene therapy, nuclease, CRISPR, targeted integration (TI), knock-in, safe harbor, homologous recombination (HR)

INTRODUCTION

Blood genetic disorders are caused by mutations in genes or in their regulatory elements that result in a dysfunctional, dysregulated, or absent protein. Conventional gene therapy approach consists of the addition of a functional copy of a mutated gene to patients’ cells using viral vectors, such as adeno-associated virus (AAV) (Mingozzi and High, 2011) and lentivirus (LV)-derived vectors (Naldini, 2011). These modified viruses can deliver the transgene expression cassettes encoded in their genome to the cell nucleus, where the genetic information is used. This gene replacement strategy is mutation-independent and thus can benefit patients with the same condition regardless of their genotype.

Despite its remarkable success for ex vivo and in vivo treatment of several monogenic disorders (Dunbar et al., 2018), there are still major hurdles to overcome to improve therapeutic outcomes and treat challenging monogenic (e.g., hemoglobinopathies, immunodeficiencies, and congenital anemias) as well as multifactorial blood diseases (e.g., cancer, autoimmune, and infectious disorders). Apart from vector-specific issues such as immunogenicity and tropism (Masat et al., 2013; Colella et al., 2018), which are beyond the scope of this review, classic gene replacement has a major limitation: it is hard to faithfully re-create characteristics of endogenous promoters and gene-specific regulation within the context of a viral vector. Tissue-, developmental-, and stimulus-specific gene expression requires the complex interaction of different genomic elements (promoters, enhancers, and silencers) that can be located in distant regions of the genome and span several kilobases (Schoenfelder and Fraser, 2019).

AAV vectors are small viruses (~4.7 kb), limiting the choice of regulatory elements to include in the expression cassette, especially when delivering large transgenes (Li and Samulski, 2020). Moreover, they persist mainly as episomes in non-dividing cells and are progressively lost through cell division (Nakai et al., 2001; Ehrhardt et al., 2003; Bortolussi et al., 2014)—a major obstacle.
for treating infantile disorders and tissues undergoing rapid proliferation (e.g., hematopoietic and epithelial cells). On the other hand, LV have larger cargo capacity (~8kb), stably integrate in the genome, and persist through cell replication (Naldini et al., 1996), but they carry the intrinsic risk of insertional mutagenesis and oncogene transactivation (mainly when strong promoters/enhancers are present (Cavazzana et al., 2019; Bushman, 2020)). In addition, their semi-random integration (Schroder et al., 2002) results in transduction mosaicism and heterogeneous transgene expression due to chromatin position effects (Chen et al., 2017; Vansant et al., 2020), making therapeutic levels harder to reach.

When combining AAV and nuclease, both transgene expression cassettes and genomic integration sites contribute to the corrective strategy, dramatically expanding therapeutic possibilities. Primarily, targeting a functional copy of a gene to its endogenous locus, under the control of its own promoter and in the right chromatin context, can result in physiological expression and minimize genotoxic integrations. Alternatively, transgenes can be targeted to safe integration sites or specific genomic elements of interest to engineer cells with novel functions, further improving safety and increasing potential applications of gene replacement/addition therapy (Cox et al., 2015).

Sequence-specific endonucleases (such as ZNF, TALEN, or CRISPR/Cas9) (Gaj et al., 2016) can induce genomic DNA double-strand breaks (DSB) in proximity to pathological mutations and activate cellular DNA repair pathways to correct them. The inclusion of short single-stranded oligodeoxynucleotide (ssODN) donors is a simple and effective approach for precise correction of single-nucleotide mutations (DeWitt et al., 2016; De Ravin et al., 2017; Romero et al., 2019). Although their short size currently limits their application for diseases caused by multiple pathological variants (e.g., β-thalassemia, ~300 different mutations across the β-globin locus), technological advances in long ssODN synthesis would most likely expand their therapeutic potential (Praetorius et al., 2017; Roth et al., 2018).

DSB generated by endonucleases can also facilitate integration of therapeutic transgenes to selected genomic locations (targeted gene replacement). AAV has a tendency to integrate at pre-existing chromosomal breaks that provide free DNA ends for non-homologous end joining (NHEJ) (Miller et al., 2004). To increase efficiency, specificity, and precision of integration, homology arms derived from genomic regions flanking the target site are introduced on each side of the AAV cassette with the aim of leveraging the homologous DNA repair pathway (Hirata et al., 2002). Although effective in proliferating cells, homologous recombination is quite inefficient in quiescent hematopoietic stem cells (HSC) and postmitotic cells or tissues (Nishiyama, 2019; Shin et al., 2020). Therefore, alternative DNA repair mechanisms based on NHEJ or microhomology-mediated end joining (MMEJ) are now being investigated (Suzuki et al., 2016; Banan, 2020). In both cases, AAV are the gold-standard DNA delivery system for gene-targeted integration in vivo (Li et al., 2011) and ex vivo (Wang et al., 2015), though the exact molecular mechanism underpinning this process remains unknown (Deyle and Russell, 2009).

**INTEGRATION STRATEGIES**

Selecting a suitable genomic site for transgene integration depends on many factors, such as the expression level required, the target cells/tissue, and the disease to be treated.

We have subdivided integration sites in four groups according to functional characteristics: (i) endogenous promoters, when promoterless transgenes are inserted under the control of endogenous enhancers/promoters; (ii) safe genomic harbors, when transgenes and their promoters are integrated into genomic regions that allow robust expression without affecting cell physiology; (iii) disease modifier genes, when transgenes integrate into coding sequence of endogenous genes, whose inactivation benefits disease-affected cells; and (iv) specificity exchange, when transgenes are integrated into coding sequence of endogenous genes to change their function.

It is worth noting that this subdivision is only a working framework, as the same integration site can fall into two or more categories, and it is not exhaustive, as new integration strategies are described every day.

**ENDOGENOUS PROMOTERS**

**Correction of Dysfunctional Genes**

A straightforward approach for targeted gene replacement consists in inserting a functional copy of a gene downstream of its endogenous promoter. This strategy can correct most pathological mutations that are scattered along the gene body (such as substitutions and frameshift mutations), while maintaining physiological gene expression (Table 1A), which can be hard to achieve with artificial promoters used in classical gene therapy vectors (Toscano et al., 2011).

The first proof of concept was obtained using ZFN on primary T cells ex vivo to replace interleukin-2 receptor subunit gamma (IL2RG), whose mutational inactivation causes X-linked severe combined immunodeficiency (X-SCID) (Urnov et al., 2005; Bushman, 2020). X-SCID represents an ideal model for testing this approach, as correction of only a small fraction of treated cells, given their strong growth advantage, should allow expansion and restoration of T cell function in vivo.

However, for effective clinical translation, targeted gene replacement should be performed in hematopoietic stem cells (HSC), the life-long source of all the different blood progenitors. Genovese via ZFN (Genovese et al., 2014) and Schirol via CRISPR/Cas9 (Schirol et al., 2017) were the first to report successful integration of a functional copy of IL2RG gene downstream its endogenous promoter in HSC, with the idea of restoring the endogenous lineage specificity and expression level of IL2RG without the risk of insertional mutagenesis (Hacein-Bey-Abina et al., 2003, 2008). Following this example, additional strategies have been developed for many blood diseases, including thalassemia (Voit et al., 2014; Dever et al., 2016), chronic granulomatous disease (De Ravin et al., 2017; Sweeney et al., 2017), hyper-immunoglobulin (Ig) M syndrome

---

**Table 1A**

| Integration Site | Function | Strategy | Applications |
|-----------------|----------|----------|-------------|
| Endogenous Promoters | Correction of Dysfunctional Genes | Inserting a functional copy of a gene downstream of its endogenous promoter | X-SCID, thalassemia, chronic granulomatous disease |
| Safe Genomic Harbors | Correction of Dysfunctional Genes | Inserting transgenes into genomic regions that allow robust expression without affecting cell physiology | X-SCID, thalassemia, chronic granulomatous disease |
| Disease Modifier Genes | Correction of Dysfunctional Genes | Inserting transgenes into coding sequence of endogenous genes, whose inactivation benefits disease-affected cells | X-SCID, thalassemia, chronic granulomatous disease |
| Specificity Exchange | Correction of Dysfunctional Genes | Inserting transgenes into coding sequence of endogenous genes to change their function | X-SCID, thalassemia, chronic granulomatous disease |
| Integration strategies | Advantages | Disadvantages | References |
|------------------------|------------|---------------|------------|
| A Endogenous locus | Physiological transgene expression | Gene-specific strategy | Urnov et al., 2005; Lombardo et al., 2007; Li et al., 2011; Genovese et al., 2014; Voit et al., 2014; Dever et al., 2016; Hubbard et al., 2016; Schiroli et al., 2017; Sweeney et al., 2017; Kuo et al., 2018; Wang et al., 2019; Rai et al., 2020; Wang L. et al., 2020 |
| B Superactive promoters (ALB, HBA) | Accommodates different transgenes, Supraphysiological expression, Few integrations required | Partial gene disruption, Limited to non-cell autonomous disorders, Extensive validation required | Barzel et al., 2015; Sharma et al., 2015; Davidoff and Nathwani, 2016; Lacharawee et al., 2018; Chen et al., 2019; Conway et al., 2019; De Caneva et al., 2019; Ou et al., 2019, 2020; Zhang et al., 2019; Wang Q. et al., 2020 |
| C Tolerant to integration (AAVS1, CCR5, Rosa26) | Accommodates different transgenes | Artificial promoters required, Variable expression | De Ravin et al., 2016; Dez et al., 2017; Stephens et al., 2018, 2019; Gomez-Ospina et al., 2019; Scharenberg et al., 2020 |
| D Chromatin domains (NAD) | Fine gene regulation, Far from oncogenic genes | No proof-of-principle in clinically relevant models | Schenkwein et al., 2020 |
| E Disease-modifier genes (CCR5, HBA) | Improve therapeutic effect, Lower therapeutic threshold | Extensive validation required, Limited to well-known diseases | Voit et al., 2013; Weibking et al., 2018 |
| F Specificity Exchange (TCR, BCR) | Improved CAR expression and potency | Off-targets, Translocations risk (for multiple edits) | Eyquem et al., 2017; MacLeod et al., 2017; Greiner et al., 2019; Hartweger et al., 2019; Moffett et al., 2019; Voss et al., 2019 |

Scissors: nuclease; Solid arrows: promoters; Enh, enhancers; TAD, topologically associating; d, domain; Solid ovals: histone modifications; Solid squares: DNA modifications.

(Hubbard et al., 2016; Kuo et al., 2018), and Wiskott–Aldrich Syndrome (Rai et al., 2020).

Beside HSC and terminally differentiated blood cells, like B and T cells (Wang et al., 2016; Hung et al., 2018), AAV and nucleases have been the preferred method to achieve targeted transgene integration in many tissues in vivo (Suzuki et al., 2019; Kohama et al., 2020; Nishiguchi et al., 2020), especially the liver.

Li et al. were the first to demonstrate targeted gene correction in vivo by delivering ZFN and a partial F9 (coagulation factor IX, FIX) cDNA cassette with AAV8 to the liver of a humanized mouse model of hemophilia B (Li et al., 2011). While correction was performed in newborn mice, FIX expression was maintained in adults and even persisted after partial hepatectomy, demonstrating stable genomic integration. This approach was later replicated using CRISPR/Cas9 to integrate...
a hyperactive FIX variant in the mouse F9 locus (Wang et al., 2019).

Targeted gene replacement can also be combined with classical gene therapy to improve therapeutic outcome. In a neonatal mouse model of ornithine transcarbamylase (OTC) deficiency, an AAV carrying a liver-specific promoter and a human OTC transgene was integrated via CRISPR/Cas9 in the murine OTC locus (Wang L. et al., 2020). Prompt, short-term expression from episomal AAV protected newborn mice from fatal hyperammonemia crisis, whereas its genomic integration allowed long-term disease correction.

Although targeting transgenes to their genomic loci is an effective therapeutic approach, it requires the development of countless gene-tailored editing strategies. Moreover, it can be difficult to reach and correct a number of cells that is sufficient to achieve a therapeutic benefit. Finally, its efficacy is limited in the presence of deletions/inversions that affect large portions of the locus or when regulatory elements controlling gene expression are mutated.

**Over/Expression by Superactive Promoters**

Although gene-editing technologies are evolving at a fast pace, it can be challenging to correct enough cells to reach a clinical benefit even using high doses of nuclease and donor DNA, which increase chances of off-target genomic cleavage, immune responses, and donor random integration. An alternative strategy consists in “hijacking” strong endogenous promoters to overexpress therapeutic cassettes from few modified cells (Table 1B). An elegant example of this approach is the targeted integration of AAV-delivered transgenes under the control of the endogenous albumin promoter in the liver (Barzel et al., 2015; Sharma et al., 2015; Davidoff and Nathwani, 2016). Even with <1% of targeted integration events, the terrific transcriptional activity of this superactive promoter was sufficient to achieve 5–20% of FIX levels and correct bleeding in hemophilia B mice (Barzel et al., 2015). Until today, this strategy has been successfully applied in different preclinical models of hemophilia A and B (Barzel et al., 2015; Sharma et al., 2015; Chen et al., 2019; Conway et al., 2019; Zhang et al., 2019; Wang Q. et al., 2020) and metabolic disorders (Laoharaweewee et al., 2018; Conway et al., 2019; De Caneva et al., 2019; Ou et al., 2019). Importantly, this is also the first genome-editing strategy undergoing in vivo testing in humans to treat mucopolysaccharidosis I and II (NTC02702115, NTC03041324).

Although promising, this approach still presents some concerns. First, targeted integration can lower serum albumin levels (Zhang et al., 2019; Ou et al., 2020) and albumin mutations have been observed in human hepatocellular carcinoma (Cancer Genome Atlas Research Network, 2017; Rao et al., 2017). Second, long-term AAV-mediated expression of endonucleases can result in off-target editing and unwanted AAV insertions (Li et al., 2019; Breton et al., 2020; Wang H. et al., 2020). Finally, pre-existing liver conditions and immune responses against AAV vectors used to deliver transgenes or nucleases severely limit the number of eligible patients (Boutin et al., 2010; Simhadri et al., 2018).

To avoid these issues, we have recently proposed to integrate therapeutic transgenes in the α-globin locus of HSC (Pavani et al., 2020). Similar to albumin targeting, the idea is to combine the strong transcriptional output of the α-globin promoter with the abundance of transgene-expressing erythroblasts to maximize protein production, reducing the number of integration events required to reach therapeutic levels. Moreover, differently from the liver, autologous HSC can be recovered from patients and edited ex vivo before re-administration, thus circumventing immunological issues. Additional experiments in preclinical disease models will elucidate the therapeutic potential of this novel HSC platform for treating genetic diseases.

Following these examples, additional endogenous promoters with specific expression levels/patterns can be exploited for transgene expression. Although promoter hijacking has many advantages over other approaches, it is important to functionally validate the dispensability of the disrupted gene, as nuclease-mediated targeting can result in bi-allelic gene knock out, or to consider safer editing alternatives (e.g., nicking endonucleases Ran et al., 2013).

**SAFE GENOMIC HARBORS**

**Tolerant to the Integration of an Expression Cassette**

Genomic safe harbors are intragenic or intergenic regions of the human genome that enable stable expression of integrated transgenes without negatively affecting the host cell (Sadelain et al., 2011). Targeting expression cassettes to these loci is an efficient way to develop a “one-fits-all” platform to express different therapeutic transgenes using the same nuclease(s), therefore optimizing efficiency and improving safety.

By far, the most widely targeted genomic loci are AAVS1, CCR5, and Rosa26 (Table 1C).

The AAVS1 locus (chromosome 19 q13.42) was historically identified as the preferential integration site of wild-type AAV in human cell lines (Kotin et al., 1992). It encodes the PPP1R12C gene, a subunit of myosin phosphatase whose functions are not fully elucidated (Surks et al., 2003), but probably redundant (Smith et al., 2008). Stable and corrective editing of patients’ HSC at this locus has been obtained by integrating a transgene cassette with (Fanconi anemia (Diez et al., 2017)) or without an exogenous promoter (X-CGD (De Ravin et al., 2016)). It is worth noting that the AAVS1 locus is an extremely gene-rich region and, although the presence of an insulator in the promoter of PPP1R12C could shield the genome from the action of the inserted promoter/enhancer (Ogata et al., 2003; Li et al., 2009), it requires a carefully designed transgene expression cassette to avoid transcriptional perturbation of neighboring genes (Lombardo et al., 2011). Moreover, several studies showed that variable expression and promoter silencing can occur at this site in different cell types (Lamartina et al., 2000; Smith et al., 2008; Ordovas et al., 2018; Bhagwan et al., 2019; Klatt et al., 2020), thus potentially limiting transgene expression.

The CCR5 gene (chromosome 3 p21.31) encodes for the main HIV co-receptor. Since a bi-allelic null mutation of this receptor (CCR5∆32) confers HIV-1 resistance and is not
associated with any major pathology (Hutter et al., 2009), this locus was first targeted/disrupted with nucleases in T cells and HSC to provide protection against AIDS ((Perez et al., 2008; Yu et al., 2020), NCT00842634, NCT02500849, and NCT03164135) and later exploited for targeted gene addition. Therapeutic transgenes involved in lysosomal storage disorders were inserted in the CCR5 gene of human HSC, under the control of exogenous ubiquitous or tissue-specific promoters. Upon transplantation, edited HSC engrafted, differentiated, and corrected the pathological phenotype in mouse models of MPS I (Gomez-Ospina et al., 2019) and Gaucher (Scharenberg et al., 2018). The genomic location of transgene integration can change its associated domains (NAD), which are distant from protein-encoding genes with oncogenic potential and thus represent safe sites for inserting therapeutic transgenes.

The increasing knowledge of chromatin functions and dynamics (Moore et al., 2020) might soon allow us to select integration sites to obtain a certain transcriptional activity and cell/tissue/developmental specificity, as predicted by the presence/absence of certain histone marks (Talbert et al., 2019), DNA methylation, transcriptional factor binding sites, nuclear lamina interaction (Amendola and van Steensel, 2014), chromatin accessibility, and topology (Zheng and Xie, 2019; Zhang et al., 2020) (Table 1D). We can easily envision that the combination of selected chromatin locations and expression cassettes will allow fine-tuning of therapeutic transgene expression to unprecedented levels.

**DISEASE-MODIFIER GENES**

**Inactivation of Pathogen Receptors**

A disease-modifier gene alters the expression of another gene involved in a genetic/infectious disorder, therefore changing the penetrance, dominance, and severity of the disease itself (Genin et al., 2008). Novel genome-editing strategies can combine transgene expression with modulation of disease-modifier genes to improve therapeutic outcomes and provide cells with novel functions (Table 1E). Voit et al. were the first to describe the use of ZFN to integrate transgenes encoding for HIV restriction factors into the HIV co-receptor gene CCR5 (Voit et al., 2013). With this strategy, treated T cells were resistant to HIV infection thanks to the concomitant expression of protective transgenes and knockout of CCR5 (disease-modifier).

**Restoring Balance in Disease Pathways**

A second example of this approach involves β-thalassemias, a group of blood disorders caused by mutations in the β-globin gene. β-globin associates with α-globin to form adult hemoglobin (HbA, α2β2) and, when β-globin chains are absent or limiting, free α-globin precipitates causing hemolysis and ineffective erythropoiesis. Reduction of α-globin has been shown to ameliorate the β-thalassemia phenotype (Mettananda et al., 2015); hence, we and others have proposed to target the integration of a β-globin transgene into the α-globin site (disease-modifier) of HSC to simultaneously express the therapeutic gene while reducing α-globin production in differentiated erythroblasts (Table 1E) (Pavani et al.; Cromer et al.; Molecular Therapy Vol 27 No 4S1, April 2019). The full potential of this combination therapy for these and other genetic diseases will be more clear in the future (Hightower and Alexander, 2018; Rahit and Tarailo-Graovac, 2020).

While the possibility of combining gene replacement and endogenous gene regulation could attain unparalleled additive or synergic therapeutic effects, it is limited to the treatment of diseases for which a deep knowledge of the underlying molecular mechanism is available, and it requires careful examination.

**Providing Novel Functions**

Targeted integration can also provide cells with novel functions, such as a “safety-switch” for cell therapy applications. Transgene integration can be directed to inactivate an essential metabolic enzyme, the uridine monophosphate synthetase, which makes T cells dependent on supplemented uridine for their growth and survival (Wiebking et al., 2018). This approach could help therapies based on chimeric antigen receptor T cells by introducing a metabolic control of their proliferation and persistence. Further experiments are required to evaluate the clinical readiness of the approach.
SPECIFICITY EXCHANGE

A special case of gene targeting is represented by the “specificity exchange” (Table 1F). Chimeric antigen receptors (CARs) are synthetic receptors that redirect and reprogram T cells to recognize specific antigens for tumor rejection (June and Sadelain, 2018). Initially, CARs were introduced in T cells using retroviral and lentiviral vectors (gene addition), with the risk of insertional mutagenesis. In addition, these CAR-T cells had two antigen specificities, the engineered one and the physiological one encoded by the endogenous αβ T cell receptor (TCR) chains, which may induce graft-vs-host disease when allogeneic T cells are used (Torikai et al., 2012).

New CAR-T cells are generated by targeting the integration of the CAR transgene under the transcriptional control of TCR α-chain gene promoter to simultaneously achieve physiological expression of CAR and disruption of the endogenous TCR, thus maintaining only CAR antigen specificity (specificity exchange) (Eyquem et al., 2017; MacLeod et al., 2017). Overall, this strategy allows uniform CAR expression in human T cells and enhances T cell potency, outperforming conventional CAR-T cells.

A similar strategy has also been described to integrate and express a sequence encoding for a defined monoclonal antibody (Ab) of interest under the control of the heavy or light immunoglobulin chain promoter to reprogram B cells to secrete broadly neutralizing Ab against pathogens, for which no protective Ab has been isolated (Greiner et al., 2019; Hartweger et al., 2019; Moffett et al., 2019; Voss et al., 2019).

CONCLUSIONS

Over the past decades, gene therapy for blood disorders has mainly focused on the optimization of transgenes and synthetic promoters to improve expression and achieve therapeutic effects using gene replacement. However, this strategy is associated with the risk of insertional mutagenesis (LV) and episomal vector loss (AAV). The advent of the first generation of DNA endonucleases allowed the integration of transgenes in few selected genomic loci, mainly to achieve stable expression while minimizing insertional mutagenesis risk. Now, thanks to easily programmable nucleases such as CRISPR/Cas9, we have dramatically expanded our integration options and can creatively exploit different genomic locations to finely tune transgene expression or modulate disease-modifier genes to improve gene therapy outcomes.

A common strategy to target transgene integration combines nucleases with a donor DNA template (generally AAV) and leverages the homologous recombination pathway. However, before clinical translation, strict functional validation will be necessary to reduce potential adverse events associated with each individual component of this system. In particular, nucleases can induce potential off-targets (Kleinstitver et al., 2016; Carroll, 2019) and chromosomal alterations induced by on-target cleavage (Adikusuma et al., 2018; Kosicki et al., 2018; Cullot et al., 2019; Ledford, 2020); nucleases and AAV activate p53 response and trigger cell cycle arrest (Schwartz et al., 2007; Haapaniemi et al., 2018; Ihry et al., 2018); donor DNA integration can occur by different DNA repair mechanisms with outcomes sometimes difficult to predict (Canaj et al., 2019; Hanlon et al., 2019; Nelson et al., 2019); the target site needs to be functionally validated for safety and disposability (Papapetrou and Schambach, 2016).

Additional studies and further optimization of existing editing technologies will remove these hurdles and allow a broad clinical application of the described strategies to treat both monogenic and multifactorial blood diseases.

AUTHOR CONTRIBUTIONS

GP and MA wrote the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by Bayer (Hemophilia Awards Program), AFM-Telethon, INSERM, Genopole (Chaire Fondagen), the European Union’s Horizon 2020 (SCIDNET No 666908), and the Agence nationale de la recherche (ANR-16-CE18 STaHR).

ACKNOWLEDGMENTS

MA thanks the members of his laboratory at the Genethon Institute.

REFERENCES

Adikusuma, F., Piltz, S., Corbett, M. A., Turvey, M., McColl, S. R., Helbig, K. J., et al. (2018). Large deletions induced by Cas9 cleavage. Nature 560, E8–E9. doi: 10.1038/s41586-018-0380-z
Akhbar, W., de Jong, J., Pindyurin, A. V., Pagie, L., Meuleman, W., de Ridder, J., et al. (2013). Chromatin position effects assayed by thousands of reporters integrated in parallel. Cell 154, 914–927. doi: 10.1016/j.cell.2013.07.018
Amendola, M., and van Steensel, B. (2014). Mechanisms and dynamics of nuclear lamina-genome interactions. Curr. Opin. Cell Biol. 28, 61–68. doi: 10.1016/j.cceb.2014.03.003
Banan, M. (2020). Recent advances in CRISPR/Cas9-mediated knock-ins in mammalian cells. J. Biotechnol. 308, 1–9. doi: 10.1016/j.jbiotec.2019.11.010
Barzel, A., Paulik, N. K., Shi, Y., Huang, Y., Chu, K., Zhang, F., et al. (2015). Promoterless gene targeting without nucleases ameliorates haemophilia B in mice. Nature 517, 360–364. doi: 10.1038/nature13864
Bhagwan, J. R., Collins, E., Mosquera, D., Bakar, M., Johnson, B. B., Thompson, A., et al. (2019). Variable expression and silencing of CRISPR-Cas9 targeted transgenes identifies the AAVS1 locus as not an entirely safe harbour. F1000Res 8:1911. doi: 10.12688/f1000research.19894.1
Bortolussi, G., Zentilllin, L., Vanikova, J., Bockor, L., Bellarosa, C., Mancarella, A., et al. (2014). Life-long correction of hyperbilirubinemia with a neonatal liver-specific AAV-mediated gene transfer in a lethal mouse model of Crigler-Najjar Syndrome. Hum. Gene Ther. 25, 844–855. doi: 10.1089/hum.2013.233
Boutin, S., Montelliet, V., Veron, P., Leborgne, C., Benveniste, O., Montus, M. F., et al. (2010). Prevalence of serum IgG and neutralizing factors against adenovirus- associated virus (AAV) types 1, 2, 5, 6, 8, and 9 in the healthy population:
implications for gene therapy using AAV vectors. *Hum. Gene Ther.* **21**, 704–712. doi: 10.1089/hum.2009.182

Breton, C., Clark, P. M., Wang, L., Greig, J. A., and Wilson, J. M. (2020). TIR-Seq, a next-generation sequencing assay, identifies genome-wide DNA editing sites in vivo following adenov-associated viral vector-mediated genome editing. *BMC Genom.* **21**, 239. doi: 10.1186/s12864-020-6655-4

Bruckner, L., van Aresenbergen, J., Ahktar, W., Pagie, L., and van Steensel, B. (2016). High-throughput assessment of context-dependent effects of chromatin proteins. *Epigenet. Chromatin* **9**, 43. doi: 10.1186/s13072-016-0096-y

Bushman, F. D. (2020). Retroviral insertional mutagenesis in humans: evidence for four genetic mechanisms promoting expansion of cell clones. *Mol. Ther.* **28**, 352–356. doi: 10.1016/j.ymthe.2019.12.009

Cahill, M. E., Conley, S., DeWan, A. T., and Montgomery, R. R. (2018).

De Caneva, A., Porro, F., Bortolussi, G., Sola, R., Lisjak, M., Barzel, A., et al. (2019). Coupling AAV-mediated promoterless gene targeting to SaCas9 nuclelease to efficiently correct liver metabolic diseases. *Nat. Commun.* **10**, 4136. doi: 10.1038/s41467-019-09906-2

Davidoff, A. M., and Nathwani, A. C. (2016). Genetic targeting of the albumin locus to treat Hemophilia. *N. Engl. J. Med.* **374**, 1288–1290. doi: 10.1056/NEJMct1603347

De Caneva, A., Porro, F., Bortolussi, G., Sola, R., Lisjak, M., Barzel, A., et al. (2019). Coupling AAV-mediated promoterless gene targeting to SaCas9 nuclelease to efficiently correct liver metabolic diseases. *JCI Insight* **5**, 128863. doi: 10.1172/jci.insight.128863

De Ravan, S. S., Reis, A., Liu, P. Q., Li, L., Wu, X., Chou, U., Allen, C., Koontz, S., et al. (2017). CRISPR-Cas9 gene repair of hematopoietic stem cells from patients with X-linked chronic granulomatous disease. *Sci. Transl. Med.* **9**, eaah3480. doi: 10.1126/scitranslmed.aah3480

De Ravan, S. S., Reis, A., Liu, P. Q., Li, L., Wu, X., Su, L., et al. (2016). Targeted gene addition in human CD34(+)-hematopoietic cells for correction of X-linked chronic granulomatous disease. *Nat. Biotechnol.* **34**, 424–429. doi: 10.1038/nbt.3513

Dever, D. P., Bak, R. O., Reinisch, A., Camarena, J., Washington, G., Nicolas, C. E., et al. (2016). CRISPR/Cas9 beta-globin gene targeting in human haematopoietic stem cells. *Nature* **539**, 384–389. doi: 10.1038/nature20134

DeWitt, M. A., Magis, W., Bray, N. L., Wang, T., Berman, J. R., Urbinati, F., et al. (2016). Selection-free genome editing of the sickle mutation in human adult hematopoietic stem/progenitor cells. *Sci. Transl. Med.* **8**, 360ra134. doi: 10.1126/scittransmed.aah336

Deyle, D. R., and Russell, D. W. (2009). Adeno-associated virus vector integration. *Curr Opin Mol Ther.* **11**, 442–447.

Diez, B., Genovese, P., Roman-Rodriguez, F. J., Alvarez, L., Schirol, G., Ugade, L., et al. (2017). Therapeutic gene editing in CD34(+) hematopoietic progenitors from Fanconi anemia patients. *EMBO Mol. Med.* **9**, 1574–1588. doi: 10.15252/emmm.201707540

Dunbar, C. E., High, K. A., Joung, J. K., Kohn, D. B., Ozawa, K., and Sadelain, M. (2018). Gene therapy comes of age. *Science* **359**, 2aax4672. doi: 10.1126/science.aaw4672

Ehrhardt, A., Xu, H., and Kay, M. A. (2003). Episomal persistence of recombinant adeno viral vector genomes during the cell cycle in vivo. *J. Virol.* **77**, 7689–7695. doi: 10.1128/JVI.77.13.7689-7695.2003

Eyskens, 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. doi: 10.1038/nature21405

Falcon, A., Cuevas, M. T., Rodriguez-Frandsen, A., Reyes, N., Pozo, F., Moreno, S., et al. (2015). CRISPR deficiency predisposes to fatal outcome in influenza virus infection. *J. Gen. Virol.* **96**, 2074–2078. doi: 10.1099/vir.0.001165

Gaj, T., Sirk, S. J., Shui, S. L., and Liu, J. (2016). Genome-editing technologies: principles and applications. *Cold Spring Harb. Perspect. Biol.* **8**, a023754. doi: 10.1101/cshperspect.a023754

Genin, E., Feinbold, J., and Clerget-Darpoux, F. (2008). Identifying modifier genes of monogenic disease: strategies and difficulties. *Hum. Genet.* **124**, 357–368. doi: 10.1007/s00439-008-0560-2

Genovese, P., Schirol, G., Escobar, G., Tomasillo, T. D., Frittoli, C., Calabria, A., et al. (2014). Targeted genome editing in human repopulating hematopoietic stem cells. *Nature* **510**, 235–240. doi: 10.1038/nature13420

Gomez-Ospina, N., Scharenberg, S. G., Mostrel, N., Bak, R. O., Mantri, S., Quadros, R. M., et al. (2019). Human genome-edited hematopoietic stem cells phenotypically correct Mucopolysaccharidosis type. *Nat. Commun.* **10**, 4045. doi: 10.1038/s41467-019-11962-8

Greiner, V., Bou Puerto, R., Liu, S., Herbel, C., Carmona, E. M., and Goldberg, M. S. (2019). CRISPR-mediated editing of the B cell receptor in primary human B cells. *Science* **362**, 397–398. doi: 10.1126/science.abc0019

Haapianen, E., Botla, P., Persson, I., Schmierer, B., and Taipale, J. (2018). CRISPR/Cas9 gene engineering induces a p53-mediated DNA damage response. *Nat. Med.* **24**, 927–930. doi: 10.1038/s41591-018-0049-z

Hacein-Bey-Abina, S., Garrigue, A., Wang, G. P., Soulier, J., Lim, A., Morillon, E., et al. (2008). Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *J. Clin. Invest.* **118**, 3132–3142. doi: 10.1172/JCI35700

Hacein-Bey-Abina, S., Von Kalle, C., Schmidt, M., McCormack, M. P., Wulfraat, N., Leboulch, P., et al. (2003). LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science* **302**, 415–419. doi: 10.1126/science.1088547

Hanlon, K. S., Kleinsteiver, B. P., Garcia, S. P., Zaborowski, M. P., Volak, A., Spirig, S. E., et al. (2019). High levels of AAV vector integration into CRISPR-induced DNA breaks. *Nat. Commun.* **10**, 4439. doi: 10.1038/s41467-019-12449-2

Hartweger, H., McGuire, A. T., Horning, M., Taylor, J. J., Dosenovic, P., Yost, D., et al. (2019). HIV-specific humoral immune responses by CRISPR/Cas9-edited B cells. *J. Exp. Med.* **216**, 1301–1310. doi: 10.1084/jem.20190287

Hightower, R. M., and Alexander, M. S. (2018). Genetic modifiers of Duchenne and facioscapulohumeral muscular dystrophies. *Muscle Nerve.* **57**, 6–15. doi: 10.1002/mus.23953

Hirata, R., Chamberlain, J., Dong, R., and Russell, D. W. (2002). Targeted transgene insertion into human chromosomes by adenovirus-associated virus vectors. *Nat. Biotechnol.* **20**, 735–738. doi: 10.1038/nbt0702-735

Hubbard, N., Hagan, D., Sommer, K., Song, Y., Khan, I., Clough, C., et al. (2016). Targeted gene editing restores regulated CD40L function in X-linked hyper-IgM syndrome. *Blood* **127**, 2513–2522. doi: 10.1182/blood-2015-11-683235
Huneg, K. L., Meitlis, I., Hale, M., Chen, C. Y., Singh, S., Jackson, S. W., et al. (2018). Engineering protein-secreting plasma cells by homolog-directed repair in primary human B cells. Mol. Ther. 26, 456–467. doi: 10.1016/j.mther.2017.11.012

Hutter, G., Nowak, D., Mosauer, M., Ganepola, S., Mussig, A., Allers, K., et al. (2009). Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. N. Engl. J. Med. 360, 692–698. doi: 10.1056/NEJMoa0802905

Ihly, R. J., Wroring, K. A., Salick, M. R., Frías, E., Ho, D., Theriault, K., et al. (2018). p53 inhibits CRISPR-Cas9 engineering in human pluripotent stem cells. Nat. Med. 24, 939–946. doi: 10.1038/s41591-018-0050-6

Irion, S., Luche, H., Gadue, P., Fehling, H. J., Kennedy, M., and Keller, G. (2007). Identification and targeting of the ROSA26 locus in human embryonic stem cells. Nat. Biotechnol. 25, 1477–1482. doi: 10.1038/nbt1136

June, C. H., and Sadelain, M. (2018). Chimeric antigen receptor therapy. N. Engl. J. Med. 379, 64–73. doi: 10.1056/NEJMa1706199

Klatt, D., Cheng, E., Hoffmann, D., Santilli, G., Thrsasher, A. J., Brindel, C., et al. (2020). Translational gene silencing of myeloid-specific promoters in the AAVSI safe harbor locus of induced pluripotent stem cell-derived myeloid cells. Hum. Gene Ther. 31, 199–210. doi: 10.1089/hum.2019.194

Klein, R. P., Bhagwat, A. S., Haurigot, V., Doyon, Y., Li, T., Wong, S. Y., et al. (2011). Efficient recombinase-mediated cassette exchange in hPSCs to study the highly efficacious PS gene editing system corrects metabolic and neurological disease in mice. Proc. Natl. Acad. Sci. U. S. A. 108, 17009–17014. doi: 10.1073/pnas.1104242108

Klutmann, S., et al. (2018). Single AAV-mediated mutation replacement genome editing in limited number of photoreceptors restores vision in mice. Mol. Ther. 26, 1127–1136. doi: 10.1016/j.ymthe.2018.03.002

Kotin, R. M., Linden, R. M., and Berns, K. I. (1992). Characterization of a small regulatory element from chromosome 19 enhances liver-specific gene expression in human embryonic stem cells. Nat. Biotechnol. 10, 111–122. doi: 10.1038/nbt.2018.125

Krishna, K. M., Fujita, K., Miyamoto, S., Nakayama, K., and Nakazawa, T. (2020). AAV-mediated CRISPR-Cas9 system for efficient genome editing of human hematopoietic cells. Stem Cells Transl. Med. 9, 559–569. doi: 10.1002/stem.3157

Laoharawee, K., DeKelwer, R. C., Podetz-Pedersen, K. M., Rohde, M., Sproul, S., Nguyen, H. O., et al. (2018). Dose-dependent prevention of metabolic and neurological complications of Mucopolysaccharidosis type I. Mol. Ther. 26, 1424–1432. doi: 10.1016/j.ymthe.2018.05.007

Lamborghini, A., Cesana, D., Genovesi, P., Di Stefano, B., Provasi, E., Colombo, D. F., et al. (2011). Site-specific integration and tailoring of cassette design for sustainable gene transfer. Nat. Methods 8, 861–869. doi: 10.1038/nmeth.1674

Lamborghini, A., Genovesi, P., Beausjour, C. M., Collesini, S., Lee, Y. L., Kim, K. A., et al. (2007). Gene editing in human stem cells using zinc finger nucleases and integrase-defective lentiviral vector delivery. Nat. Biotechnol. 25, 1298–1306. doi: 10.1038/nbt1353

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. doi: 10.1016/j.ymthe.2017.02.005

Masat, E., Pavan, G., and Mingozzi, F. (2013). Humoral immunity to AAV vectors in gene therapy: challenges and potential solutions. Discov. Med. 15, 379–389.

Metzner, S., Gibbons, R. J., and Higgs, D. R. (2015). alpha-Globin as a molecular target in the treatment of beta-thalassemia. Blood 125, 3694–3701. doi: 10.1182/blood-2015-03-63594

Miller, D. G., Petek, L. M., and Russell, D. W. (2004). Adeno-associated virus-mediated gene delivery promotes S-phase entry-independent precise targeted integration in cardiomyocytes. Sci. Rep. 10:15348. doi: 10.1038/s41598-020-72216-y

Moffett, H. F., Harms, C. K., Fitzpatrick, K. S., Tookey, M. O., Boonyaratanakornkit, J., and Taylor, J. J. (2019). B cells engineered to express pathogen-specific antibodies protect against infection. Sci. Immunol. 4:aax0644. doi: 10.1126/sciimmunol.aax0644

Moore, J. E., Purcaro, M. J., Pratt, H. E., Epstein, C. B., Shores, N., Adrian, J., et al. (2020). Expanded encyclopedias of DNA elements in the human and mouse genomes. Nature 583, 699–710. doi: 10.1038/s41586-020-2493-4

Nakai, H., Yant, S. R., Storm, T. A., Fauss, S., Meuse, L., and Kay, M. A. (2001). Extrachromosomal recombinant adeno-associated virus vector genomes are primarily responsible for stable liver transduction in vivo. J. Virol. 75, 6969–6976. doi: 10.1128/JVI.75.15.6969-6976.2001

Naldini, L. (2011). Ex vivo gene transfer and correction for cell-based therapies. Nat. Rev. Genet. 12, 301–315. doi: 10.1038/nrg2985

Naldini, L., Blomer, U., Gallay, P., Ory, D., Mulligan, R., Gage, F. H., et al. (1996). In vivo gene delivery and stable transduction of non-dividing cells by a lentiviral vector. Science 272, 263–267. doi: 10.1126/science.272.5259.263

Nishiyama, J. (2019). Genome editing in the mammalian brain using the CRISPR-Cas system. Neurosci. Res. 141, 4–12. doi: 10.1016/j.neures.2018.07.003

Ogata, T., Kitazawa, K., and Kanda, T. (2003). Identification of an insulin in AAV-S1, a preferred region for integration of adeno-associated virus DNA. J. Virol. 77, 9000–9007. doi: 10.1128/JVI.77.16.9000-9007.2003

Ordovas, L., Boon, R., Pistoni, M., Chen, Y., Wolfs, E., Gou, W., et al. (2018). Efficient recombinase-mediated cassette exchange in hPSCs to study the hepatocyte lineage reveals AAVS1 locus-mediated transgene inhibition. Stem Cell Rep. 10:673. doi: 10.1016/j.stemcr.2018.01.034

Ou, L., DeKelwer, R. C., Rohde, M., Tom, S., Radeke, R., and St Martin, S. J., et al. (2019). ZFN-mediated in vivo genome editing corrects murine hurler syndrome. Mol. Ther. 27, 178–187. doi: 10.1016/j.ymthe.2018.10.018

Ou, L., Przybilla, M. I., Ahlat, O., Kim, S., Overn, P., Barnes, J., et al. (2020). A highly efficacious PS gene editing system corrects metabolic and neurological complications of Mucopolysaccharidosis type I. Mol. Ther. 28, 1442–1454. doi: 10.1016/j.ymthe.2020.03.018

Papapetrou, E. P., and Schambach, A. (2016). Gene insertion into genomic safe harbors for human gene therapy. Mol. Ther. 24, 678–684. doi: 10.1016/j.ymthe.2016.05.08

Pavan, G., Laurent, M., Fabiano, A., Cantelli, E., Sakkal, A., Corre, G., et al. (2020). Ex vivo editing of human hematopoietic stem cells for erythroid expression
of therapeutic proteins. *Nat. Commun.* 11:3778. doi: 10.1038/s41467-020-1 7552-3

Perez, E. E., Wang, J. M., Miller, J. C., Jouvenot, Y., Kim, K. A., Liu, O., et al. (2008). Establishment of HIV-1 resistance in CD4+ T cells by genome editing using zinc-finger nucleases. *Nat. Biotechnol.* 26, 808–816. doi: 10.1038/nbt1410

Praetorius, F., Kick, B. K., Scheller, K. L., Honemann, M. N., Weuster-Botz, D., and Dietz, H. (2017). Biotechnological mass production of DNA origami. *Nature* 552, 84–87. doi: 10.1038/nature24650

Rahit, K., and Tarailo-Graovac, M. (2020). Genetic modifiers and rare mendelian disease. *Genes* 11:3023. doi: 10.3390/genes11030239

Rai, R., Romito, M., Rivers, E., Turchiano, G., Blattner, G., Vetharoy, W., et al. (2020). Targeted gene correction of human hematopoietic stem cells for the treatment of Wiskott - Aldrich Syndrome. *Nat. Commun.* 11:4034. doi: 10.1038/s41467-020-17626-2

Ran, F. A., Hsu, P. D., Lin, C. Y., Gootenberg, J. S., Konermann, S., Trevino, A. E., et al. (2013). Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity. *Cell* 154, 1380–1389. doi: 10.1016/j.cell.2013.08.021

Rao, C. V., Asch, A. S., and Yamada, H. Y. (2017). Frequently mutated genes/pathways and genomic instability as prevention targets in liver cancer. *Carcinogenesis* 38, 2–11. doi: 10.1039/carinbgw118

Romero, Z., Lomova, A., Said, S., Miggelbrink, A., Kuo, C. Y., Campo-Fernandez, B., et al. (2019). Editing the sickle cell disease mutation in human hematopoietic stem cells: comparison of endonucleases and homologous donor templates. *Mol. Ther.* 27, 1389–1406. doi: 10.1038/s41389-019.00514

Roth, T. L., Puig-Saus, C., Yu, R., Shifrut, E., Carnevale, J., Li, P. J., et al. (2018). Reprogramming human T cell function and specificity with non-viral genome targeting. *Nature* 559, 405–409. doi: 10.1038/s41586-018-0326-5

Sadellain, M., Papapetrou, E. P., and Buxman, F. D. (2011). Safe harbours for the integration of new DNA in the human genome. *Nat. Rev. Cancer* 12, 51–58. doi: 10.1038/nrc3179

Scharenberg, S. G., Poletto, E., Lucot, K. L., Colella, P., Sheikhali, A., Montine, T. J., et al. (2020). Engineering monocyte/macrophage-specific glucocerebrosidase expression in human hematopoietic stem cells using genome editing. *Nat. Commun.* 11:3327. doi: 10.1038/s41467-020-17184-x

Schenkwein, D., Afzal, S., Nousiainen, A., Schmidt, M., and Yla-Herttuala, S. (2020). Efficient nuclease-directed integration of lentivirus vectors into the human ribosomal DNA locus. *Mol. Ther.* 28, 1858–1875. doi: 10.1016/j.mtherap.2020.05.019

Schircoli, G., Ferrari, S., Conway, A., Jacob, A., Capo, V., Albano, L., et al. (2017). Preclinical modeling highlights the therapeutic potential of hematopoietic stem cell gene editing for correction of SCID-X1. *Sci. Transl. Med.* 9:aan0820. doi: 10.1126/scitranslmed.aan0820

Schonfelder, S., and Fraser, P. (2019). Long-range enhancer-promoter contacts in gene expression control. *Nat. Rev. Genet.* 20, 437–455. doi: 10.1038/s41576-019-0105-7

Schröder, A. R., Shinn, P., Chen, H., Berry, C., Ecker, J. R., and Bushman, F. (2002). HIV-1 integration in the human genome favors active genes and local hotspots. *Cell* 110, 521–529. doi: 10.1016/S0092-8674(02)00864-4

Schwartz, R. A., Palacios, J. A., Cassell, G. D., Adam, S., Giacca, M., and Weitzman, M. D. (2007). The Mre11/Rad50/Nbs1 complex limits adenovirus infection and replication. *J. Virol.* 81, 12936–12945. doi: 10.1128/JVI.01523-07

Sharma, R., Anguela, X. M., Doyon, Y., Wechsler, D., DeKelver, R. C., Sproul, S., et al. (2015). In vivo genome editing of the albumin locus as a platform for protein replacement therapy. *Blood* 126, 1777–1784. doi: 10.1182/blood-2014-12-615492

Shin, J. J., Schroder, M. S., Caiado, F., Wyman, S. K., Bray, N. L., Bordi, M., et al. (2020). Controlled cycling and quiescence enables efficient HDR in human hematopoietic stem cells using genome-editing technologies. *Nat. Rev. Genet.* 21, 786–795. doi: 10.1038/s41576-019-0105-7

Voss, J. E., Gonzalez-Martin, A., Andraibi, R., Fuller, R. P., Murrell, B., McCoy, L. E., et al. (2019). Reprogramming the antigen specificity of B cells using genome-editing technologies. *Elife* 8:42995. doi: 10.7554/eLife.42995

Voit, R. A., Hendel, A., Pruett-Miller, S. M., and Porteus, M. H. (2014). Nucleosome-mediated gene editing by homologous recombination of the human globin locus. *Nucl. Acids Res.* 42, 1365–1378. doi: 10.1093/nar/gkt947

Voit, R. A., McMahon, M. A., Sawyer, S. L., and Porteus, M. H. (2013). Generation of an HIV resistant T-cell line by targeted “stacking” of restriction factors. *Mol. Ther.* 21, 786–795. doi: 10.1038/mt.2012.284

Wang, L., Yang, Y., Breton, C., Bell, P., Li, M., Zhang, J., et al. (2020). A mutation-independent CRISPR-Cas9-mediated gene targeting approach to treat a murine model of ornithine transcarbamylase deficiency. *Sci. Adv.* 6:eax5701. doi: 10.1126/sciadv.aax5701
Wang, L., Yang, Y., Breton, C. A., White, J., Zhang, J., Che, Y., et al. (2019). CRISPR/Cas9-mediated in vivo gene targeting corrects hemostasis in newborn and adult factor IX-knockout mice. *Blood* 133, 2745–2752. doi: 10.1182/blood.2019000790

Wang, Q., Zhong, X., Li, Q., Su, J., Liu, Y., Mo, L., et al. (2020). CRISPR-Cas9-mediated in vivo gene integration at the albumin locus recovers hemostasis in neonatal and adult hemophilia B mice. *Mol. Ther. Methods Clin. Dev.* 18, 520–531. doi: 10.1016/j.omtm.2020.06.025

Wiebking, V., Patterson, J. O., Martin, R., Chanda, M. K., Lee, C. M., Srifa, W., et al. (2018). Metabolic engineering generates a transgene-free safety switch for cell therapy. *Nat. Biotechnol.* 2020:6. doi: 10.1038/s41587-020-0580-6

Yu, S., Ou, Y., Xiao, H., Li, J., Adah, D., Liu, S., et al. (2020). Experimental treatment of SIV-infected macaques via autograft of CCR5-disrupted hematopoietic stem and progenitor cells. *Mol. Ther. Methods Clin. Dev.* 17, 520–531. doi: 10.1016/j.omtm.2020.03.004

Zambrowicz, B. P., Imamoto, A., Fiering, S., Herzenberg, L. A., Kerr, W. G., and Soriano, P. (1997). Disruption of overlapping transcripts in the ROSA beta geo 26 gene trap strain leads to widespread expression of beta-galactosidase in mouse embryos and hematopoietic cells. *Proc. Natl. Acad. Sci. U. S. A.* 94, 3789–3794. doi: 10.1073/pnas.94.8.3789

Zhang, D., Huang, P., Sharma, M., Keller, C. A., Giardine, B., Zhang, H. (2020). Alteration of genome folding via contact domain boundary insertion. *Nat. Genet.* 52, 1076–1087. doi: 10.1038/s41588-020-0680-8

Zhang, J. P., Cheng, X. X., Zhao, M., Li, G. H., Xu, J., Zhang, F., et al. (2019). Curing hemophilia A by NHEJ-mediated ectopic F8 insertion in the mouse. *Genome Biol.* 20:276. doi: 10.1186/s13059-019-1907-9

Zheng, H., and Xie, W. (2019). The role of 3D genome organization in development and cell differentiation. *Nat. Rev. Mol. Cell Biol.* 20, 535–550. doi: 10.1038/s41580-019-0132-4

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Pavani and Amendola. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.