Precision genetic engineering of hematopoiesis to treat human disease

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Genetic engineering of hematopoietic stem cells (HSC) with lentiviral vectors has been providing substantial benefit to growing numbers of patients affected by primary immunodeficiencies, hemoglobinopathies and storage disorders. Long-term follow up shows stable hematopoietic reconstitution by high numbers of corrected HSC without signs of clonal expansion or exhaustion. Precise engineering by gene editing may further improve the reach and safety of HSC gene therapy by achieving in situ gene correction or targeted transgene integration. Homology-driven editing, however, remains limiting in long-term HSC and the genetic outcome at target sites heterogenous and, for some by-products, potentially genotoxic. Template delivery by Integrase-defective lentiviral vectors rather than AAV6 and the use of lipid nanoparticles instead of electroporation may increase safety and efficiency of the procedure. Coupling selection for the intended edit and purging adverse outcomes may provide a preferred path towards clinical application of this currently unique modality enabling long-range edits. On the other hand, the emergence of base and prime editors that bypass the requirement for DNA double-strand breaks (DSB) allows editing single/few mutant nucleotides with limited activation of DNA damage response. We have shown, however, that DSBs are significantly lowered but not abrogated. Moreover, the expression of constitutive deaminase domains within the editors may impact the mutagenic load of treated cells. While these potentially genotoxic outcomes can be mitigated by optimizing expression and culture conditions, they should be better investigated and monitored in emerging clinical applications. Overall, our work should advance HSC gene therapy by a combination of transformative approaches leveraging on precision genetic engineering while alleviating the morbidity of the procedure, broadening application to several diseases and patients worldwide.