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Crispr/Cas9 HR

# CRISPR/Cas9 genome engineering in hematopoietic cells

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**The development of genome editing tools capable of modifying specific genomic sequences with unprecedented accuracy has opened up a wide range of new possibilities in targeted gene manipulation. In particular, the CRISPR/Cas9 system, a repurposed prokaryotic adaptive immune system, has been widely adopted because of its unmatched simplicity and flexibility.**
**In this review we discuss achievements and current limitations of CRISPR/Cas9 genome editing in hematopoietic cells with special emphasis on its potential use in *ex vivo* gene therapy of monogenic blood disorders, HIV and cancer.**

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## Introduction

Clustered regularly interspaced short palindromic repeats (CRISPR) are defining components of the genomes of most bacteria and archaea and are part of their adaptive immune system defending against phage and plasmid DNA infection [1]. The most widely used CRISPR/Cas9 system of *Streptococcus pyogenes* (SpCas9/sgRNA) consists of three components: the CRISPR-associated DNA cleaving endonuclease Cas9 protein (~160 kDa, ~4.2 kb), a target DNA sequence-recognizing RNA transcribed from short DNA sequences known as protospacers (crRNA), and a trans-activating crRNA (tracrRNA) required for crRNA transcription [2,3].

For genome editing, fully functional fusions between crRNAs and tracrRNAs are used as single guide RNAs (sgRNA) for Cas9 targeting to pre-specified genomic sites [4]. For target identification, SpCas9 requires a trinucleotide sequence (*i.e.* 5′-NGG) located downstream of the sgRNA called protospacer adjacent motif (PAM), which determines the exact position of DNA binding [3,5]. Unlike other designer endonucleases such as Zinc Finger Nucleases and TALENs involving DNA recognizing protein domains, CRISPR/Cas9 targeting is based on RNA/DNA base-pairing, thus circumventing the laborious design and engineering of target specific proteins consisting of concatenated proteins whose specificity is dependent on domain interactions that are difficult to predict. Overall, CRISPR/Cas9 RNA-guided nucleases (RGNs) are easy to make even by laboratories not specialized in genome engineering.

Like all the other genome editing systems, RGNs generate DNA double strand breaks (DSB) at their target sites which are repaired either by homologous recombination or by nonhomologous end-joining (NHEJ). While homology directed repair (HDR) requires a template and is precise, NHEJ re-ligates the DNA ends without requiring a template in an error prone process that is associated with random nucleotide **in**sertions and/or **del**etions (indels) [6,7]. Both mechanisms have been

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exploited for targeted gene knock-outs, gene replacements and *in situ* gene repair in hematopoietic cells [8–12].

Here we discuss CRISPR/Cas9 strategies employed for the treatment of inherited blood disorders, HIV and cancer by the *ex vivo* manipulation of hematopoietic stem and progenitor cells (HSPCs) or T-lymphocytes.

### Ex vivo *genome editing*

For *ex vivo* genome editing HSPCs or T-lymphocytes are isolated from patients by apheresis and kept in tissue culture in the presence of dedicated cytokine cocktails stimulating proliferation and preventing differentiation. Following genetic manipulation, the engineered cells are reintroduced into the patients (Fig. 1). Overall, *ex vivo* gene therapy is a well-established procedure that has been implemented in numerous clinical trials [13–17].

One of the most challenging issues of the *ex vivo* approach is gene delivery to cultured hematopoietic cells. As most current gene delivery protocols do not employ drug selection, gene transduction methods need to be good enough to yield the minimum number of functionally reconstituted cells required for a therapeutic response, which varies by disease. In instances where positive selection for gene corrected cells occurs *in vivo* (*e.g.*, X-linked severe combined immunodeficiency (X-SCID), ADA-SCID, Wiscott–Aldrich syndrome (WAS)) [18–20] significantly fewer cells are required than for diseases without selection (chronic granulomatous disease (CGD), ß-thalassemia, *etc*.).

For successful genome editing all RGN components have to be present simultaneously in the target cell. Thus, sgRNA, Cas9 and the homologous recombination template (donor template) need to be delivered either as separate molecules or in combination using dedicated expression vectors. Typically, Cas9 and sgRNA are combined on either plasmid or viral expression vectors and delivered together with oligonucleotide or plasmid donor templates by electroporation and/or infection (Fig. 2) [21,22]. The most frequently employed viral expression vectors are non-integrating integrase-deficient lentiviral vectors (IDLVs) [23,24] and adeno-associated virus vectors (AAVs) [9], both expressing RGNs only transiently in target cells. This substantially reduces genotoxic and off-target effects caused by integrating vectors and sustained RGN expression. More recently, highly efficient cell transduction protocols have been developed enabling virus free delivery of Cas9 as mRNA [10,11] or protein or as pre-assembled Cas9/sgRNA ribonucleoprotein particles (RNPs) along with oligonucleotide donor templates by electroporation [9,11,27,28]. In particular, the RNP/oligonucleotide strategy appears quite promising as it shortens the cell's exposure to Cas9 and thus reduces the frequency of off-target effects without affecting on-target mutation rates.

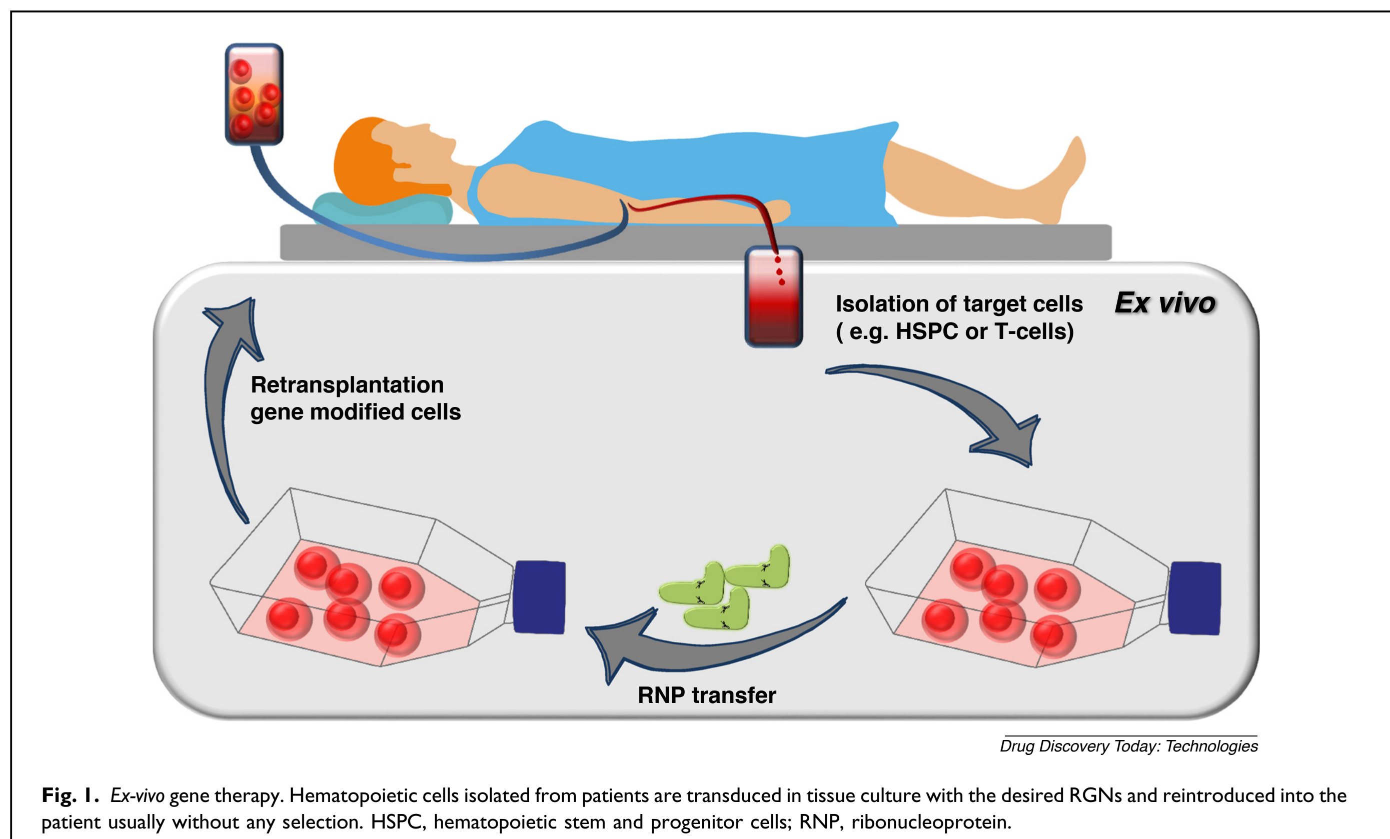


**Fig. 1.** *Ex-vivo* gene therapy. Hematopoietic cells isolated from patients are transduced in tissue culture with the desired RGNs and reintroduced into the patient usually without any selection. HSPC, hematopoietic stem and progenitor cells; RNP, ribonucleoprotein.

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