

# CRISPR-Cas9 in Gene Therapy: Much Control On Breaking, Little Control On Repairing

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## ABSTRACT

Recent advances in CRISPR-Cas9 genome editing tool have made great promises to basic and biomedical research as well as gene therapy. Efforts to make the CRISPR-Cas9 system applicable in gene therapy are largely focused on two aspects: 1) increasing the specificity of this system by eliminating off-target effects, and 2) optimizing *in vivo* delivery of the CRISPR-Cas9 DNA constructs to target cells and limiting the expression of Cas9 and gRNA to prevent immune responses. However, there is an unnoted but crucial consideration about the mode of DNA repair at the lesion caused by CRISPR-Cas9. In this commentary, I briefly highlight recent publications on *in vivo* use of the CRISPR-Cas9 system in gene therapy. I then discuss the undesired on-target DNA repair events that can occur as a result of the activity of CRISPR-Cas9. Overall, this commentary underscores the need for more study on controlled DNA repair in systems targeted with CRISPR-Cas9 genome editing tools.

Keywords: Gene Therapy, CRISPR-Cas9, DNA Repair

## COMMENTARY

Advances in CRISPR-Cas9 genome editing technology have many great promises for basic and biomedical research, as well as human therapeutics [2]. Recent reports show successful *in vivo* interrogation of genes by CRISPR-Cas9 [3][5]. It now is accepted that site-specific manipulation of genome is no longer a limitation to experiments. However, for *in vivo* gene therapy, precise genome editing can still be a bottleneck, as targeting a specific site on genome should be coupled with a controlled DNA repair.

There are well-founded concerns about the use of CRISPR-Cas9 system in gene therapy, such as the tolerance of cells towards expression of an exogenous protein such as CAS9, and specificity of the CRISPR-Cas9 system and its potential off-target site [8] [7]. Indeed, a great deal of research in the past two years has been geared towards increasing the specificity of CRISPR-Cas9 activity [4]. However, precise genome editing is not easily achieved for the purpose of *in vivo* gene therapy in which it is imperative that targeting of a specific site on the genome be accompanied by a controlled form of DNA repair.

The CRISPR-cas9 system induces site-specific double-strand breaks (DSB) DNA. Repair of the DSBs depends on one of the two repair systems: homologous recombination (HR) or non-homologous end joining (NHEJ). When repairing a mutated gene back to wild-type is desired, gene therapy often relies on HR with a provided DNA template that carries the desired sequence modification [1]. Besides the efficient delivery of vectors to the specific target cells and controlled expression of Cas9, there are considerations about the repair of the DSBs that should be taken into account while using CRISPR-Cas9 system in gene therapy. Availability of the exogenous DNA template at the time of repair, and making the HR mode of repair more favorable over NHEJ, which is naturally a cell-cycle dependent choice [6], are two limiting factors in successful *in vivo* genome editing for gene therapy.

As an example consider this gene therapy scenario: a gRNA and CRISPR-Cas9 effectively target a gene and make a DSB in a specific locus. If the homology DNA template is not available at the time of repair, or in that specific condition the NHEJ is more favorable over HR, the DSB will be repaired by NHEJ. The error prone nature of NHEJ repairs can cause insertions/deletions at the site of DSB that can: 1) make further detrimental changes in the function of the targeted gene and 2) lend the locus

non-targetable in the future.

In laboratories, *in vivo* studies in model organisms and in human cell lines do not have these limits as screening and selection helps researchers to find the desired modification. The current continuing research on genome editing by CRISPR-Cas9 should be accompanied by more studies on control of DNA repair system in targeted cells.

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