# Therapeutic Gene Editing Safety and Specificity

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**KEYWORDS**

• Gene therapy • Safety • Specific • Gene editing • Off-target

**KEY POINTS**

- Safety of gene editing is closely tied to specificity.
- Careful design of gene editing tools can improve specificity and thus safety.
- A high degree of specificity is possible with the new generation of targeted nucleases.
- Assessing the impact of gene therapy tools during their design, study, and clinical use is essential.

## INTRODUCTION

Therapeutic gene editing is advancing at an ever-increasing pace. As the list of diseases that can be treated or potentially cured with gene editing grows, it is imperative to dedicate time and energy to the topics of the safety and specificity of these technologies. This article is dedicated to a discussion of this topic in a broad sense with examples to detail how these issues affect various aspects of modifying the genetic code of patients.

As discussed in the opening article of this issue, expectations have always been high for the potential of gene therapy but early forays into its clinical application had unexpected consequences. (See Kohn DB's article, "Historical Perspective on the Current Renaissance for Hematopoietic Stem Cell Gene Therapy," in this issue.) These early setbacks in the implementation of gene therapy caused the research community and the public at large to take pause and consider the safety of this fledgling field. All involved realized that this was new and unexplored territory. Unexpected consequences can and do occur with new medical technologies. It is important to remember that expectations today may be higher for these pursuits than previous eras of medical exploration. Modifying genetic code is fundamentally different from the study of chemicals to kill a particular bacterial strain or slow the growth of a tumor. Rather than externally manipulating the biology of organisms or aberrant cells, the aim is to alter the

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blueprint of the disease-associated cells to restore or modify their function. Like a surgeon pinpoints a physical defect based on accepted anatomical function and makes repairs, so too are oncologists starting with a map (the normal genome) and working to correct errors. Precision and safety will be expected and demanded. Trial and error will only be acceptable to a point.

Before proceeding, a brief discussion of the stewardship of the human genome is prudent. The most fundamental elements of what defines being human are beginning to be modified. This requires an enormous amount of public support and trust. Although there is clearly support for the treatment and cure of genetic diseases, history shows that these technologies can also be misused. Part of safely developing these tools is considering the consequences of their misapplication. The pursuit or even the perception of the application of gene therapy for eugenics or genetic discrimination could be devastating. Care should be taken in the selection of disease targets and even in the words chosen to describe the diseases to be treated. Respect should be given to historic and cultural diversity. Maintaining a sense of transparency and being open to discussion of the practical impacts of genetic modifications are important elements to maintaining the integrity of gene therapy.

## SAFETY

Safe manipulation of the human genome is paramount to gene therapy because the intended effect of gene therapy is a permanent modification of cell function. Thus, unintended modifications that alter cell function may have long-lasting consequences.

The last decade has seen the rapid introduction of new tools, including zinc finger nucleases, homing endonucleases, transcription activator-like effector nucleases (TALENs), and RNA-guided nucleases that allow for the targeted modification of cellular genomes. The unifying activity for all of these tools is their nuclease activity, which is the ability to bind a specific sequence anywhere in the genome and introduce a DNA double-strand break (DSB). Once a DSB is generated, repair occurs through one of two basic types of mechanisms: nonhomologous end joining (NHEJ) or homology-directed repair, such as homologous recombination (HR). With enzymatically generated DSBs, NHEJ will typically lead to seamless religation of the break. However, NHEJ may introduce insertions or deletions at the DSB at appreciable frequencies, which can be useful for disrupting gene expression or function, or modifying regulatory functions mediated by the targeted sequences. HR involves the repair of a DSB using a repair template with homology to the sequences flanking the cut site. This template can either be endogenous, such as from a sister chromatid, or may be exogenously introduced. Thus, in addition to simple disruption of the targeted region, HR can be used to introduce complex engineered genetic elements.

When the genome is edited with therapeutic intent, it is an attempt to generate controlled genetic damage and the native repair mechanisms of the cell are relied on to repair the damage. In the safe translation of gene editing to a patient population, it is worth considering that induced genetic damage in the form of chemotherapy and radiation for has been used for decades. The underlying principle of cancer treatment is inducing genetic damage in a fashion that is toxic to cancer cells but does not overwhelm the repair mechanisms of healthy cells. An assessment of chromosomal instability has recently been shown to help predict the survival of a patient receiving chemotherapy and/or radiation.[1] Attempts to concentrate the genetic damage to sites of disease, such as administering intrathecal chemotherapy for central nervous system malignancies or using proton beam therapy to narrow the radiation window are steps toward tissue-level specificity but the impact on treated cells is genome wide.