selectively targeting macrophages. This intranasal vaccination method may offer exciting possibilities as a needle-free strategy since humoral responses (IgG levels) were also elevated [12]. Clearance issues are also relevant for chronic dosing, since the 'Trojan horse' should be eliminated subsequent to the delivery of the endogenous biomolecule at its desired site of action. Last but not least, the enhanced permeation retention (EPR) issues of these biopolymers at the nanoscale due to the limited lymphatic drainage in tumors should also be borne in mind.

This opinion article provides us with good evidence for 'drug hunters' globally to be inspired by and take lessons from the physicochemical properties of biopolymers and the reproducible responses of hitchhiking endogenous growth factors, cytokines, and other biomolecules. However, until exhaustive basic and applied research is undertaken on the intrinsic and extrinsic properties of polymers in complex 'patient-specific', modeled biological milieu and correlated with a defined therapeutic outcome, the classical bioconjugation-based active targeting (for example, the conjugation of monoclonal antibodies to drugs targeting cancer cells) is here to stay.

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Science & Society

Providing Appropriate Risk Information on Genome Editing for Patients

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Genome editing, represented by CRISPR/Cas9, facilitates somatic and germline gene modifications in many species, including humans. However, one of key issues, off-target mutation deserves special consideration prior to clinical applications. We herein discuss the importance of risk information on genome editing for obtaining legitimate patient consent and social acceptance.

The Uncertainty of Risk in Medicines Involving Gene Modification

According to the Declaration of Helsinki, all medical research involving humans must be preceded by careful assessment of predictable risks. Moreover, the risks must be minimized and be continuously monitored. The clinical development of new drugs has been well established, and is primarily based on phenotypic manifestations. However, this is not the case for gene modification technology because the results of gene modification remain *in vivo* for a prolonged period of time. In retrospect, there were twists and turns in the 2210 previous gene therapy trials[i]. Tragedies occurred in early trials [1]. In 1999, a gene therapy trial to restore missing ornithine transcarbamylase resulted in the death of a volunteer due to the inflammatory responses elicited by a directly delivered adenoviral vector. Moreover, several years after an *ex vivo* gene therapy to treat X-linked severe combined immunodeficiency (X-SCID), four out of ten infants who received $CD34^+$ cells with transduced *IL2RG* developed T cell leukemia due to retroviral insertions near proto-oncogenes such as *LMO2* [2]. This side effect also led to the death of a volunteer. Such catastrophic events after gene therapy underscore the need for a cautious approach in clinical trial involving gene modification, particularly in the early stages.

Genome editing technology, such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9, far more efficiently disrupt endogenous genes, repair mutations, and add exogenous genes at target sites in the human genome and potentially provide a myriad of clinical benefits. Indeed, the safety of the infusion of autologous T cells with *CCR5* modified by ZFNs has been already demonstrated in patients with HIV [3]. Among the genome editing systems, CRISPR/Cas9 is the most user-friendly due to the easiness of separate guide

RNA (gRNA) preparation, and because it facilitates simultaneous editing of several sites across the genome [4]. However, genome editing has some technical hurdles. Most notably, genome editing could induce DNA double-strand breaks (DSBs) and create large-scale genomic alterations such as chromosomal translocations in addition to small DNA insertions or deletions (indels) at non-target sites (off-target mutations) [5–7]. Such unwanted genetic changes might affect the health of volunteers enrolled in a clinical trial, potentially discrediting this biotechnology in society [8]. Although researchers are attempting to reduce the likelihood of off-target mutations [5–7], there is, at present, no clear consensus regarding the extent to which off-target effects should be detected in clinical application [9].

We herein argue the significance of clarifying and providing risk information on genome editing in order to obtain legitimate informed consent from patients.

Major Technical Issues in Genome Editing

Genome editing frequently results in high-fidelity genetic modifications at target sites because site-directed nucleases are introduced into somatic cells or the germline. However, the nucleases could simultaneously create off-target DSBs, depending on the selection of the target site and the design of the targeting molecules such as gRNA in the case of CRISPR/Cas9 [5–7] (Figure 1). Off-target DSBs could induce large-scale genomic alterations such as translocations, inversions, and large deletions other than small indels of various lengths, including point mutations [5–7]. Although such unintentional genetic changes might result in loss of function, or could lead to gain of function, or serious side effects including tumor formation due to mechanisms such as *TP53* disruption [8,10]. Moreover, the microinjection of endonucleases into animal zygotes results not only in systemic gene modifications but also mosaic modifications in the resultant organism [8,11]. However, large-scale genomic changes and mosaicism can be detected through modalities such as array comparative genomic hybridization and the genotyping of biopsied cells, respectively (Figure 1).

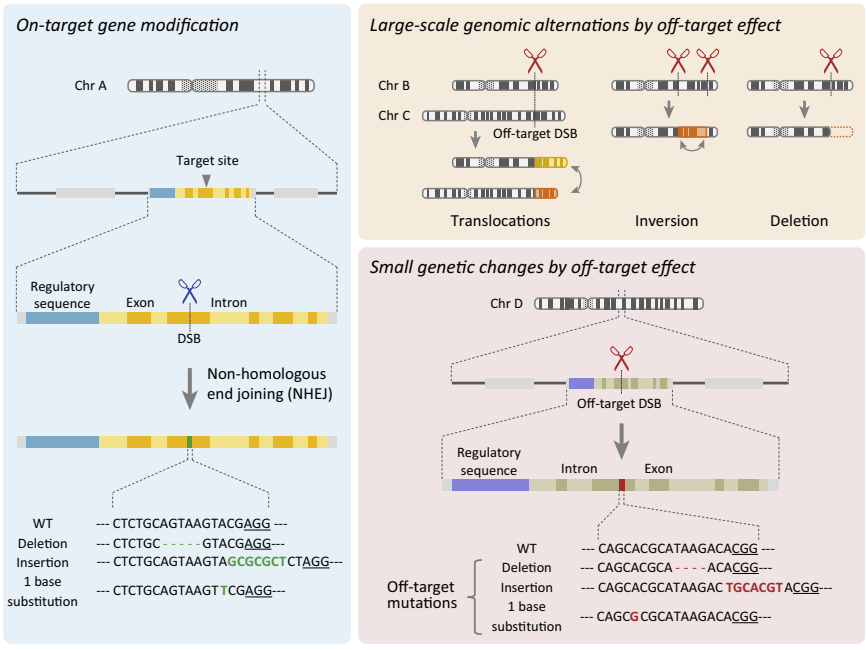

Figure 1. On-Target Gene Modification and Off-Target Effects in Genome Editing. Genome editing can efficiently modify a DNA sequence at a target site. Simultaneously, the nucleases may cause off-target effects across the genome. Specifically, the nucleases can create double-strand breaks (DSBs) at non-target sites. Although off-target DSBs could induce chromosome translocations (by creating concurrent DSBs at two loci), inversions and large deletions, such large-scale genomic alterations can be readily detected. By contrast, off-target mutations could lead to small insertions or deletions (indels) of various lengths, including point mutations, which may be found in the exons, introns, regulatory regions, or at other locations. Since such small mutations may be difficult to detect, potential off-target sites should be carefully identified prior to the clinical application. Underlined sequences (NGG) denote proto-spacer adjacent motif.

With regard to the detection of small off-target mutations in genetically modified cells, whole-exome sequencing, which analyzes all of the protein-coding regions (approximately 1% in the human genome: ~30 Mb, split across ~180 000 exons), might be an efficient and cost-effective method [5]. Meanwhile, the need for whole-genome sequencing might be proposed on the grounds that promotors and terminators impact gene expression. However, can a whole-genome analysis distinguish such small genetic changes from a single nucleotide polymorphism (SNP) or spontaneous mutations that occur during cell culture?

Genome Editing Medicine

There are three primary concepts in genome editing-based medicine (Figure 2). In *ex vivo* somatic editing therapy, the safety of the infusion of autologous T cells with *CCR5* disrupted by ZFNs (except one serious adverse event due to a transfusion reaction) has been demonstrated in 12 patients with HIV [3]. However, the *CCR5*-disrupted T cells were not investigated regarding the possible existence of off-target mutations in the clinical trial [3]. In the preclinical research, the probability of off-target mutations was rigorously estimated to be 5.39% in *CCR2*, ~1/20 000 in *ABLIM2* and there was no evidence of indels at any remaining sites (detection threshold: ~1 in 10 000