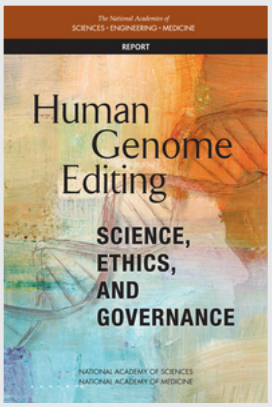

Human Genome Editing: Science, Ethics, and Governance

DETAILS

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Somatic Genome Editing

The use of human genome editing to make edits in somatic cells for purposes of treating genetically inherited diseases is already in clinical trials. Somatic cells contribute to the various tissues of the body but not to the germline, meaning that, in contrast with heritable germline editing (discussed in Chapter 5), the effects of changes made to somatic cells are limited to the treated individual and would not be inherited by future generations. The idea of making genetic changes to somatic cells, referred to as gene therapy, is not new,[1] and considerable progress has been made over the past several decades toward clinical applications of gene therapy to treat disease (Cox et al., 2015; Naldini, 2015). Hundreds of early-stage and a small number of late-stage trials are under way (Mullin, 2016), although only two gene therapies have been approved as of late 2016 (Reeves, 2016). Existing technical approaches to gene therapy are based on the results of extensive laboratory research on individual cells and on nonhuman organisms, establishing the means to add, delete, or modify genes in living cells or organisms. Prospects for future applications of gene therapy have recently been greatly enhanced by improvements in genome-editing methods, particularly the development of nuclease-based editing tools (see Chapter 3).

This chapter begins by providing background information on human somatic cell genome editing, including definitions of key terms. It then summarizes the advantages of genome editing over traditional gene therapy and

[1]Gene therapy denotes the replacement of faulty genes or the addition of new genes to cure or improve the ability to fight disease.

earlier approaches, and briefly reviews the repair methods—homologous and nonhomologous—used for nuclease-based genome editing. Next is a discussion of potential human applications of somatic cell genome editing. Scientific and technical considerations and ethical and regulatory issues are then examined in turn. The chapter ends with conclusions and recommendations. Additional scientific and technical detail on methods for genome editing are provided in Appendix A.

BACKGROUND

Genes, Genomes, and Genetic Variants

All humans contain two sets of genes inherited from their parents; each of these sets of genes is called a genome and is packaged into 23 chromosomes. The **haploid** (single) human genome is around 3 billion (3×10^9) base pairs long, and the two inherited genomes in each somatic cell (**diploid**) encode the information required for the assembly and functioning of a person's cells and body throughout life. Although people speak of the human genome, each genome differs from any other at many positions (around 1 in 1,000 base pairs, or about 3 million positions), and these genetic differences contribute to what makes individual humans unique (The 1000 Genomes Project Consortium, 2015). Many of these variations probably have little or no effect, but some affect the expression and/or functions of genes. Within the human genome lie approximately 20,000 genes that encode proteins, the molecules that actually build human cells and bodies, plus many other DNA elements that control when, where, and how much each gene is expressed (Ezkurdia et al., 2014). Some variants in genes can change the properties of the proteins they encode, while other genomic variants can affect the expression of genes. Such variants influence the color of hair or eyes, blood type, height, weight, and many other individual features, although most human traits are affected by interactions among multiple genes. Furthermore, other influences, such as diet, exercise, education, and environment, have major impacts by interacting with a person's genetic makeup.

Many of the variations in genomic sequences arise from alterations in the sequences of base pairs that arise during **replication** (copying) of the DNA during cell division (one can think of them as typographical changes). These alterations occur continually at a certain rate, and although cells have mechanisms for proofreading and correcting (editing) such changes, some escape the proofreading process and persist. Furthermore, the frequency of DNA alterations can be increased by radiation (e.g., by ultraviolet rays in sunlight or by cosmic or X-rays) or by environmental chemicals (e.g.,

cigarette smoke and other carcinogens). As mentioned, many of these variants have little or no effect, but others have positive or deleterious effects. This process of variation in human genomes has been going on since before humans evolved as a separate species and continues to this day. Evolution relies on this continual generation of variants—those that are advantageous are selected for, whereas those that are deleterious are selected against. Whether a particular variant is advantageous or deleterious, however, can vary with the context and may be a consideration in deciding whether to edit variants for clinical benefit.

Genetically Inherited Diseases

One primary impetus for interest in possible clinical applications of the recent advances in genome editing is the possibility that they provide new avenues for treating and preventing human disease. One such possible use is in the treatment of genetically inherited diseases, thousands of which are known.[2] Certain deleterious variants can be inherited from one or both parents, while others can arise de novo in the embryo rather than being inherited from either parent. The pattern of inheritance varies with the nature of the variant. If a variation that causes loss of function in a gene is inherited from one parent, it often has no evident effect, because the unaltered variant inherited from the other parent is sufficient to provide the function needed. Geneticists refer to this mode of inheritance as **recessive.** Recessive gene variants usually (but not always) have little or no effect in the so-called **heterozygous** state, when two different variants are present in the fertilized egg (zygote) and in the subsequent child and adult. That is, a person generally will not have the disease caused by a recessive deleterious gene variant unless that variant is inherited from both parents. If both parents are heterozygous, each having one copy of a deleterious variant, each of their children will have a 25 percent chance of inheriting two copies of that variant—the so-called **homozygous** state. In that case, there is no functional variant available, and the consequence may be a genetically inherited disease. Many examples of this phenomenon exist (e.g., certain forms of severe combined immunodeficiencies, such as bubble boy disease, as well as sickle-cell anemia and Tay-Sachs disease).

Other variants may actually produce medical problems even when present in a single copy despite the presence of a functional gene variant. Such variants, called **dominant,** produce deleterious effects even in the heterozygous state. A clear example is Huntington's disease, in which a single copy of a dominant disease-causing variant produces late-onset disease.

[2]OMIM, https://www.omim.org (accessed January 10, 2017); Genetic Alliance, http://www.diseaseinfosearch.org (accessed January 25, 2017).

Some inherited diseases, such as certain forms of hemophilia, which affect blood clotting, involve genes that are present on the X chromosome (so-called **X-linked**). Because men have only one X chromosome, whereas women have two, a single abnormal X-linked hemophilia gene in a man will lead to the disease being manifest, whereas women with just one deleterious variant will be carriers of the altered gene, usually without having bleeding symptoms (so called silent **carriers**).

Adding to the complexity of understanding genetic disorders is the observation, noted above, that some variants may be either deleterious or advantageous depending on the context. Probably the best known example is sickle-cell disease, which is caused by a variation in one of the genes encoding hemoglobin, the protein that carries oxygen in red blood cells. If the sickle hemoglobin variant is inherited from both parents (homozygous), it causes the hemoglobin protein to aggregate under certain conditions, leading to deformation of the red blood cells into a sickled shape that interferes with blood circulation, causing multiple difficulties and much pain and impairment of normal tissue functions. Heterozygous individuals (heterozygotes) who inherit just one sickle gene variant have few if any signs of disease and are known as carriers since they carry the sickle-cell variant and can pass it on to their children. It turns out that heterozygosity for this variant makes carriers somewhat resistant to malaria parasites that infect their red blood cells. That is, the sickle-cell variant provides a significant survival advantage in areas where malaria is present, and for that reason has been selected for and is relatively prevalent in such areas such as Africa, India, and the Mediterranean, where carriers are more common than in other areas. There are other examples of such balanced selection based on heterozygous advantage, balanced against the disadvantage of inheriting two disease-associated variants.

Finally, it is important to note that most human diseases are thought to be affected by genetic variants in multiple genes, with each variant having only a minor effect on disease progression. Thus, while the prospect of human genome editing to treat genetically inherited diseases has great appeal in some cases—for example, those in which a single gene can be clearly identified as causal—that is not true of the majority of common human diseases.

ADVANTAGES OF GENOME EDITING OVER TRADITIONAL GENE THERAPY AND EARLIER APPROACHES

Gene therapy is the introduction of exogenous genes into cells with the goal of ameliorating a disease condition. This is most efficiently done using viral vectors that take advantage of a virus's natural ability to enter cells. The viral vectors are used to introduce a functional transgene and

compensate the malfunction of an inherited mutant gene (gene replacement) or to instruct a novel function in the modified cells (gene addition). The vectors also include exogenous transcriptional regulatory sequences (promoter) to drive transgene expression. Because viral vectors have a limited cargo capacity, both the transgene and the promoter have to be modified from the natural version present in the genome and may thus fail to properly recapitulate physiological expression patterns. According to the choice of vector and type of target cells, the genetic modification may be transient, long-lasting, or permanent. Permanent modification is achieved using lentiviral or gamma-retroviral vectors that physically insert into the genome of the infected cells (integration). However, because insertion is semi-random, it may affect the function and expression of genes at or nearby the insertion site, thus representing a potential risk (insertional mutagenesis). Currently, tremendous progress is being made in gene therapy because of improved viral vectors, particularly lentiviral and recombinant adeno-associated viruses (rAAV), and these strategies are being intensively investigated in the clinic. However, despite the fact that remarkable benefits are being reported in most treated patients (Naldini, 2015), more flexible and precise genetic modifications, such as those made possible by targeted genome editing, are needed to further improve the safety of gene therapy and broaden its application to the treatment of more diseases and conditions.

Until the past decade, attempts to use genome modification in the treatment of genetically inherited disease, also called **gene targeting,** were made by introducing a DNA template carrying the desired sequence into a cell population in culture, and then either allowing insertion at a random location or relying on rare homologous recombination events to incorporate that template sequence at an intended location in the genome. The DNA template generally was introduced into the cell using such systems as recombinant plasmids (small circular pieces of DNA) or viral vectors, which take advantage of a virus's natural ability to enter cells. The rare cells that acquired the desired sequence then had to be genetically selected and clonally expanded. Despite the limitations of this approach, the importance of gene targeting as an experimental tool is reflected in the broad use of homologous recombination to modify yeast, vertebrate cell lines, or even mice to genetically dissect a wide range of biological processes (Mak, 2007; Orr-Weaver et al., 1981).

The frequency of successful gene targeting using these older strategies ranged from 10^{-6} (1 in 1 million cells) for plasmid DNA to 10^{-2}-10^{-3} (1 in 100 to 1 in 1,000 cells) using viral vectors (such as rAAV). When scientists modify DNA with a nuclease that makes a double-strand break (DSB) at a desired location in the genome, however, the frequency of successful genome editing increases dramatically (Carroll, 2014; Jasin, 1996). Nuclease-

based systems that make targeted genetic alterations are at the root of the genome-editing technologies discussed in this report. With nuclease-based editing systems, it is now possible to cut and, consequently, modify up to 100 percent of the desired target sequence in the genome, either by small insertions or deletions introduced by the nonhomologous end-joining DSB repair, or by relying on homologous recombination to introduce a new sequence at the target site, albeit with a somewhat lower efficiency. These dramatic improvements in efficiency have enabled scientists and clinicians to consider using genome editing for a greatly expanded range of applications, including application to the treatment of diseases.

Flexibility

Nuclease-based genome editing encompasses various methods for altering the DNA sequence of a cell. This editing can achieve several types of results, depending on where in the DNA the edits are made and for what purpose. Changes that can be made with genome editing include

- targeted disruption (inactivation) of the coding sequence of a gene (gene disruption);
- precise substitution of one or more nucleotides (e.g., in situ conversion of a genetic variant to wild type or to another allelic variant);
- targeted insertion of a transgene into a predetermined site for protein-coding genes;
- targeted alterations made to non–protein coding genetic elements that regulate gene expression levels (e.g., promoters, enhancers, and other types of regulatory elements);[3] and
- creation of large deletions at chosen genome locations.

Safety and Effectiveness

Nuclease-based genome editing may abrogate the risk of insertional mutagenesis inherently associated with prior gene-replacement vectors that integrate quasi-randomly throughout the genome, although late-generation integrating vectors used today may mitigate this risk. In addition, in situ gene correction of inherited mutations using genome editing reconstitutes both the function and the physiological control of expression of the mutant gene. This provides a safer and more effective correction strategy than gene

[3]Small insertions or deletions can be created to inactivate an element; larger defined deletions can be created to remove entire elements; specific nucleotide substitutions can be made in the element; or new genetic elements can be inserted into precise locations in the genome.

replacement, in which expression of the therapeutic transgene is driven by a reconstituted artificial promoter. Such randomly inserted transgenes may fail to reproduce the physiological expression pattern faithfully, and they can be strongly influenced by the insertion site, giving rise to substantial variegation of expression among a population of transduced cells. Indeed, one of the first potential applications of ex vivo genome editing may well be stem cell–mediated correction of primary immunodeficiencies—an improvement over prior transgenic approaches in which ectopic or constitutive expression of the therapeutic gene posed a risk of cancerous transformation or malfunction. If on-target editing frequencies of clinically relevant cell types are high enough to be therapeutically useful, genome editing may eventually outperform gene replacement (traditional gene therapy) in terms of safety, provided that off-target changes do not pose similar risks by modifying genes associated with cancer.

Another potential broad application of genome editing is precisely targeted integration of a gene expression cassette into a so-called safe genomic harbor, chosen because it is conducive to robust transgene expression and allows a safe insertion that does not have a detrimental effect on adjacent genes. This approach may ensure predictable and robust expression of a therapeutic gene without the risk of oncogenesis caused by inadvertent insertional activation of an oncogene. Targeted integration into a safe harbor and in situ correction of mutations are both potentially widely applicable to stem cell–based therapies as long as the targeted cells are amenable to extensive in vitro culture selection and expansion prior to clinical use. One can envisage increasing application of these types of genome editing as the ability to grow and differentiate different types of cells in culture improves, particularly in conjunction with differentiation from pluripotent cells (Hockemeyer and Jaenisch, 2016).

Gene Disruption

A unique application of genome editing relative to standard gene therapy strategies is targeted gene disruption. Indeed, clinical testing of gene disruption using zinc finger nucleases (ZFNs) is already under way, with some indication of benefit for T-cells (Tebas et al., 2014), and this approach has recently been extended to hematopoietic stem cells (HSCs). These trials aim to disrupt expression of a cytokine receptor, C-C chemokine receptor type 5 (CCR5), which also functions as a coreceptor for HIV infection and is not essential for T-cell function, thus making the T-cells of an HIV-

infected individual resistant to viral infection.[4] Gene disruption could, in principle, also be used to eliminate a dominant disease-causing gene variant.

Accessibility

Multiple nuclease platforms have been developed or improved in the past 5-10 years, making it likely that additional such platforms will be developed in the near future. The CRISPR/Cas9 nuclease platform, developed just since 2012, has generated significant optimism among research, clinical, and patient communities and has democratized genome editing, making it usable by many more laboratories. As a result, CRISPR/Cas9 has raised awareness of genome editing as a therapeutic tool and motivated consideration of the ethical and regulatory issues associated with its use (Baltimore et al., 2015; Corrigan-Curay et al., 2015; Kohn et al., 2016). These issues are not new, however, nor are they specific to the CRISPR-Cas9 system; many of them have already been confronted and addressed in the context of earlier gene therapy and genome-editing applications.

HOMOLOGOUS AND NONHOMOLOGOUS REPAIR METHODS USED FOR NUCLEASE-BASED GENOME EDITING

Nuclease-based genome editing relies on the design of an artificial enzyme—a nuclease—to bind a specific target sequence in the genome where it creates either a DNA double-strand break or a DNA single-strand cut known as a "nick." The cell usually repairs the break through one of two major mechanisms: (1) nonhomologous end joining (NHEJ), which frequently inactivates the gene or genetic element during the repair process; or (2) homology-based mechanisms, generically described as homology directed repair (HDR). (See also Chapter 3.)

Genome editing by NHEJ creates an insertion or deletion ("indel") at the break site that alters the sequence of the edited gene. Importantly, while genome editing by NHEJ is precisely located by where the DNA break or nick is produced, it is not possible to predict the size or sequence of the resulting change in a single cell or the variability of the changes (indels) among a group of cells.

In genome editing by HDR, a DNA template is used either to create one or more nucleotide changes, perhaps to match a known human reference sequence, or to insert a novel sequence (e.g., one or more genes) at a precise

[4]There are six clinical trials involving the use of ZFNs to disrupt expression of CCR5. Three of these trials have been completed, one is ongoing, and two are currently recruiting participants. For more information, see https://www.clinicaltrials.gov/ct2/show/NCT02500849?term=zinc+finger+nuclease+CCR5&rank=1 (accessed January 10, 2017).

genomic location. In contrast to NHEJ, HDR-mediated genome editing allows scientists to predict both where the edit will occur and the size and sequence of the resulting change. Thus, HDR-mediated editing is analogous to editing a document because it enables precise changes in DNA sequence.

POTENTIAL HUMAN APPLICATIONS OF SOMATIC CELL GENOME EDITING

Genome-editing applications can be categorized based on several general features:

- **Which cells or tissue(s) are modified**—in particular, whether the modification is made in somatic cells or tissues, which do not contribute to future generations; in a germ cell or germ cell progenitor, which can result in heritable changes passed to future children; or in a zygote, in which case both somatic and germ cells would be modified. (The focus here is on somatic editing; germline editing is discussed in Chapter 5).
- **Where the editing takes place**—in the test tube, followed by return of the cells or tissues to the individual (ex vivo), or directly in the person's body (in vivo).
- **The specific goal(s) of the modification**—for example, to treat or prevent disease or to introduce additional or new traits. These goals may be achieved by modifying a pathogenic DNA variant to a known nonpathogenic variant present in human reference sequences, or by modifying a gene to a sequence other than one that is a known existing human sequence.
- **The precise nature of the modification**—simple modification of a disease-causing mutation or risk-associated allelic variant, or more a complex change, such as disruption or ectopic/overexpression of an endogenous gene or addition of a novel function that augments a biological response or establishes resistance to a disease or pathogen.

The intent of each of these modifications could be to treat or prevent a disease but could also be to modify (or, in principle, even create novel) phenotypic traits in the treated cells or tissues. It is important to note, for example, that one can use genome editing to achieve enhancement of a cellular property (e.g., secreting supranormal amounts of protein or resisting a viral infection) with the intent of curing a disease. Such cellular enhancement with intent to modify disease course needs to be distinguished from the concept of enhancement aimed at creating a desired or novel organismal feature in humans (a topic discussed in detail in Chapter 6).

Table 4-1 provides examples of the types of human diseases that might

TABLE 4-1 Examples of Potential Therapeutic Applications of Somatic Cell Genome Editing*

Disease	Inheritance/ Transmission Pattern	Ex vivo or In vivo	NHEJ or HDR Mediated Editing	Stage of Development	General Strategy
Sickle-Cell Disease	Autosomal recessive	Ex vivo (HSPC)	HDR	Clinical development	Edit to non-disease-causing variant
Sickle-Cell Disease/β-Thalassemia	Autosomal recessive	Ex vivo (HSPC)	NHEJ	Preclinical	Induction of fetal hemoglobin
Severe Combined Immunodeficiency X-linked (SCID-X1)	X-linked recessive	Ex vivo (HSPC)	HDR	Clinical development	Knock-in of full or partial complementary DNA (cDNA) to correct downstream disease-causing variants
X-Linked Hyper IgM Syndrome	X-linked recessive	Ex vivo (T-cells)	HDR	Preclinical–clinical development	Knock-in of full cDNA to correct downstream disease-causing variants
Hemophilia B	X-linked recessive	In vivo (liver)	HDR	Clinical trial*	Express clotting factor from a strong promoter
Cystic Fibrosis	Autosomal recessive	In vivo (lung)	HDR	Discovery	Edit to non-disease-causing variant

HIV	Viral infection	Ex vivo (T-cells and HSPC)	NHEJ	Clinical trial	Engineer resistance to HIV
HIV	Viral infection	Ex vivo	HDR	Discovery	Engineer constitutive secretion of anti-HIV factors
Cancer Immunotherapy	NR	Ex vivo (T-cells)	NHEJ or HDR	Conceptual through clinical trial	Engineer more potent cancer-specific T-cells by genome editing
Duchenne's Muscular Dystrophy (DMD)	X-linked recessive	In vivo	NHEJ	Preclinical	Deletion of pathologic variant to convert Duchenne muscular dystrophy (DMD) to milder Becker's muscular dystrophy
Huntington's Disease	Autosomal dominant	In vivo	NHEJ	Discovery	Delete disease-causing expanded triplet repeat
Neurodegenerative Diseases	Various	Ex vivo or in vivo	HDR	Conceptual	Engineer cells to secrete neuroprotective factors

*Current information on clinical trials is available at ClinicalTrials.gov.
NOTE: HDR = homology-directed repair; HSPC = hematopoietic (blood) stem and progenitor cells; NHEJ = nonhomologous end joining; NR = not relevant (the edits are to lymphocytes designed to kill the cancer).

be treated using somatic cell genome editing. Even though this list is not comprehensive, it highlights the broad range of potential applications.

Clear examples of how genome editing might be applied to cure disease are to use homologous recombination to change the variant that causes sickle-cell disease back to the sequence that encodes wild-type β hemoglobin (Dever et al., 2016; DeWitt et al., 2016) or correct the deficits in severe combined immune deficiencies (Booth et al., 2016). A more subtle use of genome editing to correct a disease-causing variant is to insert the wild-type DNA copy of the mRNA (complementary or cDNA) into an endogenous locus to correct downstream mutations (Genovese et al., 2014; Hubbard et al., 2016; Porteus, 2016). Concerning the liver as a target organ, it has been shown that targeted insertion of a clotting factor transgene downstream of the promoter of the albumin gene in a fraction of hepatocytes may rescue the hemophilia bleeding phenotype in mouse models (Anguela et al., 2013; Sharma et al., 2015).

Several potential applications of genome editing entail causing gene disruption, provided that the delivery of the nuclease does not lead to loss of the treated cells because of toxicity or immune rejection. Among these applications are the disruption of dominant mutations and expanded triplet repeats in some neurodegenerative diseases, such as Huntington's disease (Malkki, 2016), and the reconstitution of a functional dystrophin in Duchenne's muscular dystrophy by deletion or forced skipping of the exon carrying the disease-causing mutation (Long et al., 2016; Nelson et al., 2016; Tabebordbar et al., 2016). Other examples include disruption of an endogenous gene repressor to rescue expression of a fetal gene compensating for a defective adult form, as is currently being attempted by disrupting expression of BCL11A in the erythroid lineage; to rescue fetal globin expression to compensate for the lack of expression of adult β globin in thalassemia major; or to counteract the sickling β globin mutant in sickle-cell anemia (Hoban et al., 2016). In T-cell immunotherapy, a promising application of genome editing is single or multiplex disruption of genes that may antagonize, counteract, or inhibit the activity of exogenous cell-surface receptors introduced into T-cells to direct them against tumor-associated antigens (Qasim et al., 2017). These strategies can strongly potentiate current cell-based immunotherapy strategies, possibly overcoming current barriers that limit efficacy in most solid tumors.

SCIENTIFIC AND TECHNICAL CONSIDERATIONS ASSOCIATED WITH THE DESIGN AND APPLICATION OF GENOME-EDITING STRATEGIES

All types of genome editing involve consideration of certain parameters that together determine the efficacy and potential toxicity of a genome-

editing tool. These scientific and technical considerations inform how and why a particular approach is chosen to meet a research or therapeutic goal; they also impact the nature of the data that will be available for the regulatory evaluations that will be required for potential preclinical testing, clinical trials, review, and ongoing oversight of these methods.

Choice of Engineered Nuclease Platform

The choice of nuclease includes the platform type, which can be based on protein-DNA recognition (e.g., meganucleases, ZFNs, or transcription activator-like effector nucleases [TALENs]) or on nucleic acid base-pairing recognition (e.g., CRISPR/Cas9), and the design and generation of the components that target the intended genomic sequence. When developing protein-based DNA-binding domains that are made using zinc fingers and TAL effectors, extensive engineering and improvement are possible for each specific sequence-binding domain, such that it is difficult to make a general prediction on the performance and specificity of the overall platform. That is, for ZFNs and TALENs, optimization of performance (activity and specificity) often requires work for each nuclease that may or may not translate to another nuclease.

In contrast, when RNA-based nucleases such as Cas9 are developed, general improvements are made to the platform itself and should translate to each specific target sequence. Because the only major difference among CRISPR-Cas9 systems is the targeting guide RNA, optimization of one Cas9 nuclease often will generalize to improved performance of other nucleases. This fact has implications for the ease or speed with which genome-editing systems designed for one clinical application could be adapted to target others.

Delivery Strategy: Ex Vivo and In Vivo Genome Editing

Genome editing can be carried out **ex vivo** or **in vivo.** In ex vivo editing, it is possible to conduct a number of checks on the edited cells before they are administered to a patient because the cells are first manipulated in the laboratory. Ex vivo editing, which occurs outside the body, is suitable only for certain cell types, however. By contrast, in vivo editing allows other types of cells and tissues to be edited, but poses additional safety and technical challenges because it involves administering the genome-editing tool directly into a patient's body fluids (e.g., blood), body cavities, or organs in order to modify targeted cells in situ.

Ex vivo genome editing can be performed by isolating and manipulating a population of the intended target cells outside the body and then transplanting those cells into an individual. The source of cells can be

autologous or allogeneic: autologous cells are derived from the same individual, while allogeneic cells are derived from an immunologically matched donor. Whether the cells are sourced from the same patient or a matched donor, the administered cells often have stem cell–like properties, which may allow their self-renewal and long-term maintenance in vivo, as well as repopulation of the treated tissue with their genetically modified progeny. In some approaches, the cells can be treated in culture to induce commitment or differentiation toward a desired cell type or lineage before being administered to the patient. Otherwise, the edited cells can be differentiated somatic cells, such as short-lived or long-lived immune effector cells that are expanded and genetically modified ex vivo to enhance their activity against a tumor or infectious agent. Several somatic cell types have been isolated, genetically modified, and transplanted, including blood-forming hematopoietic (blood) stem and progenitor cells, fibroblasts, keratinocytes (skin stem cells), neural stem cells, and mesenchymal stromal/stem cells. This list likely will grow as scientific knowledge and techniques improve. An expanded repertoire of cell types has the potential to increase the range of possible ex vivo genome-editing applications.

In in vivo genome editing, the editing machinery that needs to be delivered to the cells includes the nuclease that cuts the DNA and, in the case of CRISPR/Cas9, the guide RNA that targets the editing to a specific genomic location. If HDR is intended, a homologous template is also required. Targets of in vivo genome editing may include long-lived tissue-specific cells, such as muscle fibers, liver hepatocytes, neurons of the central nervous system, or photoreceptors in the retina, but may also include rare, tissue-specific stem cells and other types of cells that cannot easily be harvested and transplanted. Relative to ex vivo approaches, however, in vivo approaches pose greater challenges with respect to efficient delivery of the genome-editing machinery to the right cells in the body, ensuring that the correct location in the genome has been successfully edited, and minimizing errors resulting from off-target editing.

Additional Considerations

A number of additional scientific and technical considerations related to both ex vivo and in vivo genome editing inform the development of human genome-editing systems.

Ability to Isolate the Relevant Cell Types

To carry out ex vivo genome editing, it is necessary first to isolate the relevant cell types from an appropriate tissue source or to generate them from pluripotent stem cells, and then to grow and modify them ex vivo and

finally administer them to the patient so that they can engraft and/or deliver the intended biological activity. There are several advantages to the ex vivo strategy: only the intended cells are exposed to the editing reagents, there is a wide choice of delivery platforms that can best be fitted to each cell type and application, and it is possible to characterize and even purify and expand the edited cells before administration. Currently this process has been established for only a few cell types, including cells that will eventually give rise to skin, bone, muscle, blood, and neurons. The range of possible ex vivo genome-editing applications will expand with the development of scientific knowledge about how to isolate additional primary cell types and derive other cell types from pluripotent cells, grow the cells ex vivo, and ultimately transplant them back into patients successfully and safely.

Ex vivo genome-editing strategies have a number of expected limitations, which are common to all attempts at culturing cells ex vivo. These limitations include the need for prolonged culture and expansion from a few cells or even a single founder cell, both of which entail the risk of accumulating mutations, as well as incurring replicative exhaustion. This issue is particularly relevant for genome editing because inducing double stranded breaks in DNA, as is required to initiate the process, may itself trigger such cellular responses as apoptosis (cell death), differentiation (changing cell type), cell senescence (aging), and replicative arrest (cells stop dividing). All of these cellular responses are detrimental to cell expansion and maintenance of pluripotency.

These limitations represent significant hurdles to ex vivo genome editing because most therapeutic applications require substantial numbers of cells for infusion. Overcoming these hurdles will require better ways to culture cells, better understanding of the safety risks associated with genomic accrual of random mutations in these settings, and reliable assays for assessing such events. Additional hurdles are the ability to fully control the commitment and differentiation of cells in culture and their purification from the source pluripotent cells. This is an important consideration because administration of immature cells may be associated with a risk of tumorigenesis or failure to integrate functionally within the tissue. Despite these limitations, ex vivo genome editing has the advantage that cells with the desired alteration can be selected and the accuracy of the alterations validated before transplantation to the patient.

Ability to Control Biodistribution of the Genome-Editing Tool

Additional considerations for in vivo genome editing are linked to the choice of the delivery platform for the editing machinery because this choice impacts the extent, time course, and in vivo biodistribution of the genome-editing tool. This consideration has major implications for poten-

tial efficacy, acute and long-term toxicity, and immunogenicity and even the risk of unintentional editing of germ cells. Efficient editing of the intended genomic site usually requires a high level of intracellular nuclease expression, even though this often can be for only a short time to prevent excess toxicity and off-target activity. Whereas short-term, high expression of the genome-editing nuclease can be obtained relatively easily for cells cultured in vitro, it is more challenging in vivo. Finally, in an in vivo setting there could be unintentional (inadvertent) modification of the germ cells or primordial germ cells; therefore, preclinical development of in vivo editing should address the risk of modification of germ cells resulting in heritable changes that could be passed on to future generations and minimize this potential risk in humans enrolled in clinical trials.

In general, the risk of germline transmission associated with the administration of ex vivo genome-edited cells is likely to be low if one can show that the editing reagents do not remain associated with the treated cells and are not shed in active form at the time of administration. In these conditions, nonclinical studies of germline transmission may not be necessary. On the other hand, in vivo administration of editing reagents would require assessment of their potential biodistribution to the gonads and activity on germ cell genomes. These parameters will be strongly influenced by the delivery platforms used and the timing and route of delivery. When viral vectors are used to deliver the nuclease, the preclinical studies might take into consideration accumulating knowledge from animal and human studies concerning the potential of these vectors to reach germline cells. Preclinical studies in animal models such as nonhuman primates could be designed to monitor both the biodistribution of the vector/vehicle as well as the activity of the nuclease in cells from off-target tissues, including the gonads.

A suggested approach to studying the potential of germline transmission in such nonclinical models would be to follow a decision tree, in which a positive finding triggers the next level of investigation. One could first investigate the presence of the reagent and/or genomic signs (indels) of its activity in the gonads; next identify their actual occurrence in germ cells isolated from the positive gonads; and then determine the transient or sustained occurrence of this finding and, eventually, the transmission of the genetic modification to the viable progeny of the treated animals. Molecular assays could be designed to track the occurrence of indels at the intended or surrogate nuclease target sites, provided that such sites exist in the genome of the species used for the study with sufficient affinity for the nuclease to support the sensitivity of the assay. Many limitations exist when conducting such studies in surrogate animal species, as already discovered for several gene therapy products, including the low sensitivity of the available assays, species-specific differences in vehicle biodistribution and access to the gonadal cells, and the general difficulties of testing transmission to

the female versus male germline. Because of these limitations, regardless of the outcome of nonclinical biodistribution data, contraceptive measures are usually recommended (if meaningful or applicable) for patients undergoing in vivo gene therapy in clinical trials, at least for the expected time of clearance of the administered vector/vehicle from the body fluids, and usually extended to encompass at least one cycle of spermatogenesis (approximately 64-74 days in men). Testing of semen can be done at various points during this time interval; if samples are positive, the testing should continue, and the respective regulatory authorities should be notified. On the other hand, there are currently no noninvasive means of monitoring women for germline transmission.

Ability to Limit Immune Response to Delivery Vectors or Genome-Editing Proteins

In vivo delivery of proteins and nucleic acids is currently done with either of two types of platforms. The first is based on chemical conjugates (lipo- and/or glyco-complexes) that provide short-lived but relatively inefficient expression across multiple different tissue types, although advances have been achieved in targeting specific cell types, such as liver (Yin et al., 2014). This approach can expose therapeutically irrelevant cell types in the patient to the potential toxicity of the nuclease. The second type of platform relies on viral vectors that can provide robust and tissue-specific expression, but they also are frequently long-lived and more likely to provoke an immune response. Self-complementary rAAV8 vectors (scAAV), for example, have been shown to mediate continued expression of the engineered nuclease. Sustained nuclease expression increases the risk of DNA damage and genotoxicity, with subsequent potential risk of widespread (albeit possibly slow) cell death or malignant transformation of the patient's cells. Moreover, all current formulations of editing machinery contain elements that are derived from proteins of common microbial pathogens, which could trigger primary or secondary immune responses in treated individuals. As has been well documented in viral gene therapy studies, immune recognition of viral vector proteins may lead to rapid and complete clearance of cells that have received the editing machinery, which eliminates the benefit of the treatment. The risk of clearance of the edited cells is exacerbated by preexisting immunity and by the extent and duration of expression of the antigen.

Ability to Make Genome Edits in Nondividing Cells

Another major hurdle for both ex vivo and in vivo editing is that targeted insertion of a DNA sequence into postmitotic cells, such as neurons, is not

feasible because of their low or absent homologous recombination activity. In contrast, NHEJ, which is active in nondividing cells, has been harnessed mainly for the generation of indels to inactivate a gene. However, NHEJ can, with modifications to the methods, be used to generate site-specific gene insertions (e.g., Maresca et al., 2013; Suzuki et al., 2016). Most recently, it was reported that one of these methods, homology-independent targeted gene integration, or HITI, allows targeted knock-in of DNA sequences in dividing cells (e.g., stem cells) and most importantly, in nondividing cells (e.g., neurons) both in vitro and in vivo (Suzuki et al., 2016).

In vivo genome editing is a highly sought-after application that has been shown to be feasible and potentially therapeutic in some mouse models. Substantial challenges to its translation to the clinic remain, however, at least in the current modalities of administration. Also considering the well-known difficulties of predicting immune response in animal models, stable expression of nucleases, despite being apparently well tolerated in some animal models, may not be the preferred route to clinical development.

Assessment of the Activity and Specificity of Genome Editing

Each targeting nuclease can be characterized by the *efficiency* and *specificity* of DNA cleavage. Efficiency can be relatively easily measured (by sequencing the targeted site). Specificity reflects on-target versus off-target site activity, which also can be measured by various assays, each with advantages and disadvantages (see Appendix A for details). While whole-genome sequencing could be the gold standard for analyzing single cells or clones, the depth of this sequencing is not sufficient to assess the off-target spectrum in populations of cells.

Comparing Off-Target Editing Rate with the Natural Mutation Rate of the Human Genome

It is important to note that accurate assessment of the specificity of a genome-editing approach requires that mutations created by the genome-editing process be distinguished from those that occur spontaneously throughout a life span. The natural error frequency of normal genome replication varies among sites in the genome but is approximately 10^{-10} per base per round of DNA replication. Because each human cell contains approximately 6 billion DNA base pairs, even the naturally low error rate means that DNA replication can be expected to generate, on average, approximately one or a few de novo mutations in each round of cellular replication. Thus, as cells proliferate, they naturally accumulate mutations at this rate. In addition to this background mutation frequency, a significant amount of DNA damage results from normal environmental

exposures such as radiation, oxidative stress, and DNA-damaging agents in the environment. A direct comparison between the mutation frequency generated by a genome-editing nuclease and the spontaneous mutation frequency has not yet been conducted, but results from this type of analysis are likely to depend on the specific nuclease in question and on which cell type is examined. The error rate of nuclease technologies continues to improve and may at some point, if it is not already, be less than the spontaneous mutation frequency.

Measuring Efficiency and Specificity for Each Delivery Platform

For genome-editing applications, the system (nuclease and targeting sequences) must be delivered inside cells. Because the choice of delivery platform determines the extent, level, and time course of expression of the genome-editing machinery, it affects the efficiency and specificity displayed in a given set of experimental conditions and furthermore determines the toxicity and immunogenicity profile. In addition, several intrinsic features of the chosen delivery platform (DNA, RNA, or protein; delivery mechanism) also influence its potential toxicity (see Table A-1 in Appendix A). These effects usually are due to normal innate target-cell responses to exogenous molecules, and they often are stronger for DNA—especially DNA plasmids—than for RNA or proteins. The innate responses to viruses may vary with virus and cell type: usually they are very low for AAV or lentivirus in human somatic cells (Kajaste-Rudnitski and Naldini, 2015), with the exception of some immune cell types, such as dendritic cells and macrophages, which have a large complement of built-in viral sensors and may trigger interferon and inflammatory responses (Rossetti et al., 2012). The purity and composition of reagents (plasmid versus linearized DNA, mutant bases in RNA, high performance liquid chromatography [HPLC] purification of components) also can play a significant role.

Finally, the frequency with which the intended target sequence and related sequences occur in the genome and the local chromatin environment at the target site also can influence the efficiency and specificity of a genome editing approach. All the factors mentioned above are likely to vary according to the treated cell type and modality (ex vivo versus in vivo). Moreover, the ratio of on- to off-target activity also is affected by the intrinsic biology of the targeted cell type, including differences in cell-cycle status, DNA-damage responses, and repair capability.

Preclinical Studies to Assess Efficiency and Specificity

In the development of human genome-editing applications, preclinical studies are undertaken to establish the activity and specificity of each editing

nuclease system. The design of these preclinical studies is influenced by the choice of target cells and experimental conditions, and the results should be viewed as providing relative rather than absolute values. An additional caveat is that most of these preclinical studies measure nuclease activity and specificity over a large population of cells, among which nuclease expression will vary. Because the ratio of on- to off-target activity also varies with nuclease expression level, the cells with higher nuclease expression may have a less favorable ratio since the on-target activity will saturate, while activity at off-target sites becomes more evident. On the other hand, cells with lower expression may exhibit a more favorable ratio because activity is evident mainly at the intended target site. This consideration suggests that the dose dependence of on- and off-target rates be considered as part of the process of validating a genome-editing approach.

Assessment of nuclease specificity will continue to evolve as scientific knowledge and techniques improve. From an operational standpoint as of this writing (late 2016), however, the following represents a reasonable approach to conducting this assessment:

- Use both bioinformatics and unbiased screens to identify potential off-target sites (see Appendix A).
- Use deep sequencing of both cell lines and the primary target cell type to determine the frequency of indels at both on- and off-target sites (validation).
- Evaluate validated off-target sites for potential biological effects, and eliminate nucleases that generate off-target activity at sites that could be predicted to have biologic effects. It should be noted that most off-target sites identified to date lie in non-protein-coding regions of the genome, making their functional importance difficult to assess.
- Use assays that measure gross chromosomal integrity, such as karyotyping, single-nucleotide polymorphism (SNP) arrays, and translocation assays. These assays are limited in being relatively insensitive.
- Use diverse functional assays of the target cells of interest to measure the risk of clonal dominance and to assess the actual feasibility, efficiency, and toxicity of the genome-editing manufacturing process.

It is important to note that to develop a genome-editing approach for clinical use, it may not be necessary or feasible to conduct comprehensive efficiency and specificity studies performed at high-enough sensitivity to capture all possible off-target edits. Ongoing work in standard gene therapy, for example, has indicated that uncontrolled lentiviral insertions, which cause even more disruptive changes than nonhomologous repair of a double-strand break, may be relatively safe and well tolerated in several types of cells and tissues. This is true even when large numbers of insertions

(up to 10^8 or 10^9 per patient) are introduced. A further consideration is that the off-target activity is dependent on the sequence. Much of the early preclinical testing aimed at establishing targeting efficacy and specificity has been carried out in nonhuman organisms, especially mice. However, the genomes of humans and mice are sufficiently divergent that assessment of the specificity of engineered nucleases in the genomes of mice or other rodents may have somewhat limited predictive power for the same genome-editing approach in humans.

Summary

In summary, genome editing is already being incorporated into somatic gene therapy approaches, and such applications are likely to increase. Genome-editing strategies are in competition with other therapeutic approaches, including small molecule therapies; biologics; and most notably other gene therapy approaches, such as lentiviral vectors and rAAV vectors used for gene replacement. In the end, therefore, each strategy will need to be evaluated against the others in terms of efficacy, risk, cost, and feasibility.

ETHICAL AND REGULATORY ISSUES POSED BY SOMATIC CELL GENOME EDITING

In most respects, somatic cell genome editing will be developed with the benefit of gene therapy's robust base of technical knowledge, and within the existing system of regulatory oversight and ethical norms that have facilitated the current research and clinical development of somatic cell and gene therapy around the world, including the Australia, China, Europe, Japan, and the United States (see Chapter 2). These regulatory systems include a wide range of preclinical models and study designs to support the clinical development of therapies based on edited cells, as well as a roadmap for first-in-human clinical testing and eventual marketing.

Regulatory Oversight in the United States

As described in Chapter 2, clinical testing of somatic cell genome editing could not begin in the United States without the Food and Drug Administration's (FDA's) first having approved an Investigational New Drug (IND) application, and the clinical protocol would require institutional review board (IRB) approval and ongoing review (FDA, 1993). In addition, review by the National Institutes of Health's (NIH's) Recombinant DNA Advisory Committee (RAC) informs the deliberations of the FDA and IRBs and provides a venue for public discussion. Other countries have

similar pathways, as described in Chapter 2, albeit with some variations in the stage of research at which a cell-based therapeutic can be marketed and the terms under which it can be withdrawn.

The question of approval for clinical use hinges largely on identifying when benefits may be expected to outweigh risks when a therapy is used as labeled and as intended (Califf, 2017). Clinical trial data are increasingly reviewed within a structured framework that identifies need, alternatives, areas of uncertainty, and avenues for risk management.[5] According to former FDA Commissioner Robert Califf,

> FDA product review teams must weigh scientific and clinical evidence and consider conflicting stakeholder and societal perspectives about the value of benefits and the tolerability of risks. They must consider the existence and effectiveness of alternative treatments, disease severity, risk tolerance of affected patients, and potential for additional insight from postmarket data. Such decisions require seeking the appropriate balance between high-quality evidence and early access, between benefit and risk, between protecting the US public and encouraging innovation that may improve health outcomes. (Califf, 2017)

Approval of a gene therapy may depend upon how carefully risks and benefits can be monitored once it enters clinical use. On this topic, the FDA has issued an influential (though nonbinding) guidance for gene therapy trials that would have relevance to genome-editing trials as well (FDA, 2006). Long-term follow-up is not always required, for example, when preclinical data on factors such as vector sequence, integration, and potential for latency demonstrate that long-term risks are very low. But when long-term risks are present, "a gene therapy clinical trial must provide for long-term follow-up observations in order to mitigate those risks" (FDA, 2006, p. 1). Without such a plan for long-term follow-up observations, the risks would be unreasonable and (presumably) the trial not approvable. Where merited, the guidance suggests a 15-year period of posttrial contact, observation, and physical exams (though this can be shortened based on such factors as vector persistence, or when subjects are predicted to have only short-term survival). Prior to enrolling, subjects must give voluntary, informed consent to long-term follow-up, and while they may withdraw at any time, it is hoped that they will comply.

Once approved by the FDA for particular populations and indications, gene-based therapies would be subject to postmarket monitoring and adverse event reporting, and special warnings added. The products would be

[5]Structured Approach to Benefit-Risk Assessment in Drug Regulatory Decision-Making, PDUFA V Plan (FY 2013-2017). Draft of February 2013. http://www.fda.gov/downloads/ForIndustry/UserFees/PrescriptionDrugUserFee/UCM329758.pdf (accessed January 30, 2017).

withdrawn completely if shown to be unsafe or ineffective. In addition, postmarket risk evaluation and mitigation strategies (REMSs), such as requiring physicians to have special proficiency or requiring patients to be entered into a registry, could be required if significant safety concerns would preclude approval absent these extra controls.

Off-label use of cells subjected to genome editing would be legal in the United States, in Europe, and in other countries, and is probably to be expected with respect to patient populations (e.g., if approved for adults, use might well be extended off-label to pediatric populations) or for varying degrees of severity of the disease indication.[6] The prospect of off-label use has led to speculation about uncontrolled expansion of the technology into uses that are unsafe, unwise, unnecessary, or unfair. And it is true that off-label use, while an important aspect of innovative medicine, can at times lead to uses that lack a rigorous evidentiary basis. But the specificity of these edited cells may limit the range of off-label uses for unrelated indications more than is the case with many drugs.[7] While one might imagine a cell therapy based on genome editing for muscular dystrophy being of possible interest to those with healthy muscle tissue who wish to become even stronger, other examples are more difficult to envision, at least for the near future. This point is of particular relevance to concerns about uses that go beyond restoration or maintenance of ordinary health (discussed in Chapter 6) because the specificity of edited cells makes such applications less likely at this time.

Several technical challenges faced in moving somatic genome editing toward clinical testing have already been met by conventional somatic gene therapy. Concerning ex vivo strategies, they are based on modifying human cell types and thus can be tested only in in vitro culture models or upon xenotransplant of the modified cells into immunocompromised mice. These studies interrogate cell viability, biodistribution, and biological function in vivo, including self-renewal, multipotency, and clonogenicity, all crucial features of stem cells. In vivo strategies may require preclinical testing of toxicity and biodistribution in nonhuman primates, including evidence that unintentional modification of the germline does not occur. Indeed, the field of gene therapy has determined that in vivo approaches that would lead to unintentional modification of the germline should not be permitted. Note, however, that most assays of germline transmission have low sensitivity, and thus a certain degree of uncertainty may have to be managed in considering clinical development and regulation.

[6]The FDA recently held a public hearing to discuss its regulations and policies on manufacturer communications about unapproved or off-label uses of medical products, including cell-based therapies (FDA, 2016a).

[7]Communication, FDA, December 15, 2016.

Several guidance documents have been published by regulatory authorities in the United States and Europe and by the International Conference on Harmonisation (ICH) to illustrate the general principles for investigating and addressing the risks for inadvertent germline integration of gene therapy products in nonclinical studies, and to provide considerations for minimizing this potential risk in humans enrolled in clinical trials (EMA, 2006; FDA, 2012a; ICH, 2006). Such guidelines could be suitably adapted to the design of preclinical studies of somatic genome-editing strategies.

In an effort to speed the development of regenerative medicine, a new public–private partnership has been launched. The International Standards Coordinating Body was established

> to advance process, measurement, and analytical techniques to support the global availability of cell, gene, tissue-engineered, and regenerative medicine products, and cell-based drug discovery products. Creating standards creates a more uniform compliance environment and addresses and assists in future efforts for harmonization internationally of the regulatory framework for submissions across the globe.[8]

The sectors of activity include genetic modification of cells, with specific mention of standards for measuring off-target events in genome editing (Werner and Plant, 2016).

Regulating Somatic Genome Editing by Approach and Indication

An ethical and regulatory assessment of future somatic genome-editing applications may depend on both the technical approach to the editing and the intended indication. Like traditional gene therapy, somatic genome editing could be used to revert an underlying genetic mutation to a variant not associated with disease, which would result in a fraction of the targeted cells regaining normal function. Somatic genome editing also could be used to engineer a cell so that its phenotype differed from that of a normal cell and was better able to resist or prevent disease. For example, a cell could be changed so that it made above-normal amounts of a protein, or so that it was resistant to a viral infection. Both ex vivo and in vivo approaches to genome editing could be applied to treat or prevent a disease. In addition, genome editing could be used to alter a trait not associated with disease (see Chapter 6).

Regardless of the final framework used to assess human somatic cell genome-editing applications, it is vital that the regulatory oversight mechanisms have sufficient legal authority and enforcement capability to identify

[8]See http://www.regenmedscb.org (accessed January 10, 2017).

and block unauthorized applications. To date, the existing structures have been successful in preventing unauthorized applications of gene therapy, and the current framework provides guidance on key elements. Although human genome editing may be somewhat more difficult to control than traditional gene therapy because technical advances have made the editing steps easier to perform, the cellular manipulations and delivery of edited cells to the patient continue to demand high-quality laboratory and medical facilities, which generally will ensure that regulatory oversight is in place.

Preventing Premature or Unproven Uses of Genome Editing

The issue of unregulated therapy has been particularly problematic in the field of stem cell/regenerative medicine, with rogue entities around the world making scientifically unfounded claims about stem cell therapies and profiting from desperate patients (Enserink, 2016; FDA, 2016b; Turner and Knoepfler, 2016). In part this is due to some of the past unduly optimistic statements about the near-term prospects of regenerative medicine, in part to the presence of unregulated jurisdictions, and in part to some resistance—at least in the United States—to the regulatory authority of the government. In the United States, federal courts have confirmed the FDA's jurisdiction over the use of manipulated cells, but this is still the subject of some confusion.[9] Edited cells—particularly those taken from a patient and then returned to that patient—may engender the same confusion about whether this is a regulated product or merely the practice of medicine, and the regulatory authority needs to be made clear from the outset. Overall, then, regulatory bodies need the legal authority, leadership commitment, and political support to apply their legal powers to halt the marketing of therapies that use human genome-editing products that have not undergone regulatory review and approval (Charo, 2016a). With regard to stem cell therapies, there has been considerable concern about the absence of vigorous use of enforcement powers by the FDA (Turner and Knoepfler, 2016), although Italy's experience with closing down one clinic has illustrated the level of legal and political power needed to do this (Margottini, 2014).

Special Considerations Associated with Genome Editing in Fetuses

In certain situations, either the most effective or the only approach would be to attempt to edit the somatic cells of a fetus prior to delivery. Diseases for which these special circumstances might apply include those that are multisystemic or have an extremely early onset that would make postnatal intervention too late to benefit the child or are extremely chal-

[9] *U.S. v. Regenerative Sciences*, 741 F. 3d 1314 (D.C. Cir 2014).

lenging from a technical standpoint. In addition, because of the tremendous developmental plasticity of the fetus, fetal editing might be more effective than postnatal editing in certain circumstances. An example would be attempting to revert a disease-causing variant that affects every neuron in the brain.

In a more general sense, the therapeutic editing process could be carried out ex vivo in a scenario in which cells could be harvested from the fetus, edited outside the body, and then transplanted back into the fetus. Currently, established methods for isolating and transplanting autologous fetal cells are available for a limited number of cell types, but the range of cell types is likely to increase in the future.

Therapeutic editing in fetuses also could be performed in vivo, in which case the editing machinery would be delivered to the fetus to modify cells in situ. As noted above, the in situ correction of a disease-causing variant early in development has the potential to be more effective than postnatal in vivo editing, when many organ systems are more fully developed. In utero stem cell therapy has been tried (with limited success) (Couzin-Frankel, 2016; Waddington et al., 2005), so the general concept of in utero therapy with emerging areas of medicine has already undergone some ethical analysis. And an International Fetal Transplantation and Immunology Society has been formed, which holds annual meetings to review prospects and progress for fetal gene therapy.[10]

Although fetal genome editing has potential advantages, at least two special ethical issues would need to be addressed: special rules for consent (see Chapter 2) and the increased risk of causing heritable changes to the germline by causing modification of germ cells or germ cell progenitor/stem cells.

With regard to consent, key issues have been addressed by existing oversight mechanisms, fetal surgery has already been used in clinical care, and in utero fetal gene therapy is attracting increasing interest (McClain and Flake, 2016; Waddington et al., 2005). The risk/benefit calculation is shifted relative to a postnatal or adult intervention, with the degree of risk to which a fetus can be subjected being strictly limited when there is no prospect of medical benefit to the future child. When such benefit is possible, however, the more usual standards for risk/benefit balance apply. Decisions about fetal surgery have been made with the understanding that the pregnant woman has the ethical and legal authority to give informed consent. In the United States, as in other countries, maternal consent is required (Alghrani and Brazier, 2011; O'Connor, 2012), and when research is aimed at maternal health as well, maternal consent alone is sufficient.[11]

[10]See http://www.fetaltherapies.org (accessed January 30, 2017).

[11]Research Involving Pregnant Women or Fetuses, 45 CFR, Sec. 46.204.

In the United States, however, NIH-funded research is subject to special regulations set forth at 45 CFR Part 46, Subpart B, and paternal consent (if available) also is required if the research holds the prospect of benefit solely to the fetus. Even when not funded by NIH, many studies in the United States employ these same rules.

A second issue is the challenge of assessing whether unintended germline editing has occurred if in vivo somatic editing is attempted in a fetus. A key feature of germline cell development is that the primordial cells that will give rise to germ cells are sequestered from somatic cells at key developmental points. Before this sequestration of germline and somatic cells occurs or has been finalized in early development, germline cells might be edited as efficiently as would be the desired somatic cell targets. As a result, there could be a higher risk of unintentional edits to germline cells early in fetal development compared with performing the same intervention later in fetal development. It might be possible only to assess postnatally whether editing of germ cells or germ cell progenitors had occurred, at which time it would be too late to change the outcome.

CONCLUSIONS AND RECOMMENDATIONS

In general, there is substantial public support for the use of gene therapy (and by extension, gene therapy that uses genome editing) for the treatment and prevention of disease and disability (Robillard et al., 2014). Human genome editing in somatic cells holds great promise for treating or preventing many diseases and for improving the safety, effectiveness, and efficiency of existing gene therapy techniques now in use or in clinical trials. While genome-editing techniques continue to be optimized, however, they are best suited only to treatment or prevention of disease and disability and not to other less pressing purposes.

The ethical norms and regulatory regimes already developed for gene therapy can be applied for these applications. Regulatory assessments associated with clinical trials of somatic cell genome editing will be similar to those associated with other medical therapies, encompassing minimization of risk, analysis of whether risks to participants are reasonable in light of potential benefits, and determining whether participants are recruited and enrolled with appropriate voluntary and informed consent. Regulatory oversight also will need to include legal authority and enforcement capacity to prevent unauthorized or premature applications of genome editing, and regulatory authorities will need to continually update their knowledge of specific technical aspects of the technologies being applied. At a minimum, their assessments will need to consider not only the technical context of the genome-editing system but also the proposed clinical application so that anticipated risks and benefits can be weighed. Because off-target events

will vary with the platform technology, cell type, target genome sequence, and other factors, no single standard for somatic genome-editing specificity (e.g., acceptable off-target event rate) can be set at this time.

RECOMMENDATION 4-1. Existing regulatory infrastructure and processes for reviewing and evaluating somatic gene therapy to treat or prevent disease and disability should be used to evaluate somatic gene therapy that uses genome editing.

RECOMMENDATION 4-2. At this time, regulatory authorities should authorize clinical trials or approve cell therapies only for indications related to the treatment or prevention of disease or disability.

RECOMMENDATION 4-3. Oversight authorities should evaluate the safety and efficacy of proposed human somatic cell genome-editing applications in the context of the risks and benefits of intended use, recognizing that off-target events may vary with the platform technology, cell type, target genomic location, and other factors.

RECOMMENDATION 4-4. Transparent and inclusive public policy debates should precede any consideration of whether to authorize clinical trials of somatic cell genome editing for indications that go beyond treatment or prevention of disease or disability.