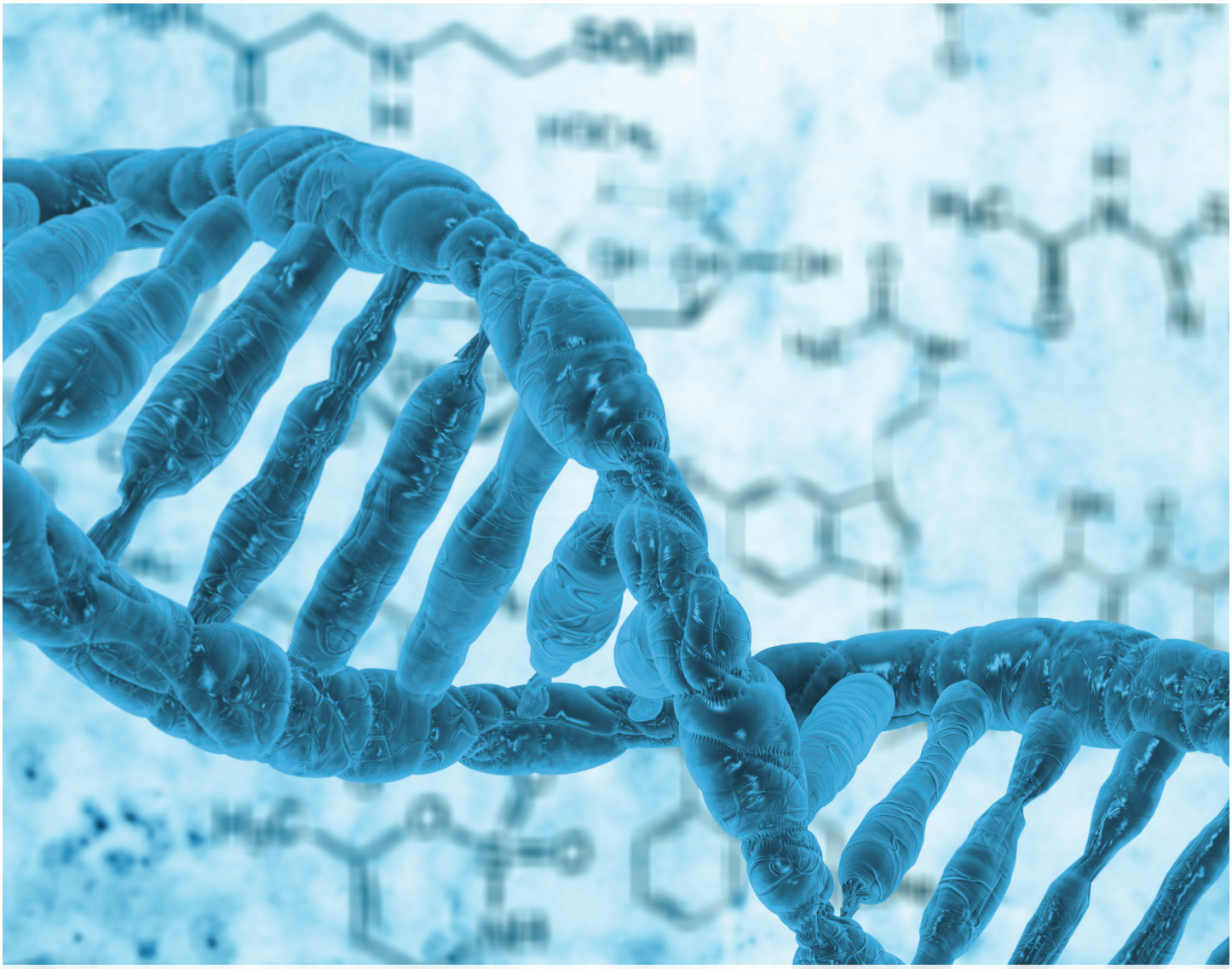

Gene Therapy and Genomic Editing: Understanding Basic Concepts

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Recent advances in recombinant DNA technology—especially "CRISPR" techniques—have raised public interest in the potential for GENOME EDITING to change the future of medicine, and potentially, of human society at large. An essential distinction for understanding evolving issues is the difference between genetic interventions intended to affect only an individual subject versus approaches intended to alter GERM LINE cells in ways that may be passed on to future generations. This paper provides a brief background in these topics so readers can better understand the roles of Gene Therapy and Genome Editing in clinical trials and pharmaceutical development.

Human Gene Transfer, Gene Therapy and Genome Editing.

Genetic information is passed from parent to offspring encoded in DNA (DeoxyriboNucleic Acid). Within each living cell, genetic instructions are processed by coordinated mechanisms involving DNA and RNA (RiboNucleic Acid). DNA and RNA are both nucleic acid molecules. Most molecular biology research involving NIH funds is subject to the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (the *NIH Guidelines*). The *NIH Guidelines* provide a specific technical definition of Human Gene Transfer (HGT) intended to encompass experimental interventions that i) incorporate engineered nucleic acids, and ii) are designed to make lasting changes in the way that

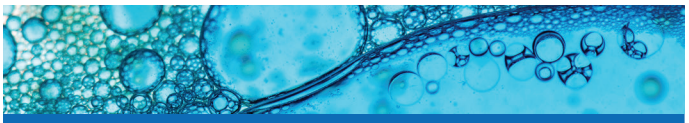
Gene Therapy, Gene Editing and Genomic Editing

In general, the structural machinery of a cell is made up of polypeptides (proteins), which are composed of amino acids. The information corresponding to the amino acid sequence of each protein is encoded, in DNA, in the respective ***gene*** for that protein. The DNA composing the genes (the coding DNA) is only a small fraction of the total DNA in a human cell– the remaining DNA (noncoding DNA) includes important information but does not specifically encode protein sequences. The total DNA information within a cell, including the coding and noncoding DNA, is called the ***genome***. The DNA of the human genome is packaged in ***chromosomes***.

For decades, the term ***gene therapy*** has been applied to approaches where an engineered DNA sequence representing a gene is introduced into human cells for the purpose of treating disease; in this context, gene therapy is not targeted to alter the native DNA sequence of the disease-associated gene in the subject's chromosome. In clinical applications, ***gene editing*** refers to one of several newer approaches that can alter the native sequence of DNA of the target gene on the chromosome. CRISPR-based technology is a form of gene editing. Because some editing applications involve changes to noncoding DNA, the term ***genomic editing*** is favored over the term *gene editing* when the broadest possible definition is desired.

genetic information is processed in targeted cells. According to this definition, HGT includes interventions involving genetically-engineered vaccines or stem cells, for example, in addition to interventions designed to treat inherited genetic diseases.

Cells that produce sperm and ova are called ***germ line*** cells; all other cells of the body are ***somatic cells***. In adult humans, the germ line cells are the only cells from which genetic information is passed on to the next generation. Changes to the DNA of a person's liver, blood, or neuronal cells affect only that person, whereas changes to a person's germ line cells could affect that person's children as well as subsequent generations. Because of consequences potentially extending for generations, intentional modification of **germ line DNA** with recombinant technology entails more risk and controversy than simple somatic gene therapy. Obviously future generations cannot give informed consent, and the ethical challenges surrounding such an intervention remain to be addressed.



Research involving deliberate recombinant alteration of the DNA of germ line cells in human subjects or in sperm, ova or zygotes intended to produce viable human embryos, is not currently permitted in any jurisdiction. Limited and cautious permission to conduct genomic editing in embryos not destined to produce viable births has been recommended.

In theory, there are two ways that the DNA of germ line cells may be artificially altered: first, the DNA of adult germ line cells could be changed such that sperm and ova produced from those cells carry through the process of fertilization and transmit the new DNA sequence to a resulting zygote. Secondly, DNA of sperm or ova, or of a zygote may be targeted during the process of artificial fertilization such that every cell of the resulting individual—including the germ cells—carries the altered genetic sequence. The former is a potential risk that must be considered as part of the risk assessment for human gene therapy trials. The latter is the most likely approach to be contemplated for human genome editing should such research be permitted in the future.

Current Human Gene Transfer Research is Individual and Therapeutic

The DNA of each person encodes a unique set of genetic information. All of the genetic information in the cells of an individual constitutes that person's *genome*. With the exception of identical twins, the genome of each person is unique. For some individuals, the unique genetic instructions in their genome result in what we recognize as a disease state. For example, when an individual lacks the genetic instructions to produce functional clotting factors, he is affected by the disease *hemophilia*. If an individual lacks genetic instructions to produce any of several essential immune system proteins, he is affected by *Severe Combined Immune Deficiency Syndrome* (*SCIDS*, a.k.a. "boy-in-the-bubble"

GloFish®, a Genetically-Enhanced Pet

Currently any ethically-permissible genetic-intervention research in human beings is undertaken only for therapeutic purposes—that is, for the purpose of treating, preventing, or curing a medically-recognized disease. From a technical standpoint however, there are no barriers to manipulating the genetics of human and nonhuman animals for nontherapeutic purposes—broadly considered genetic enhancement. In the USA many pet stores stock strains of zebra fish and tetras that have been genetically engineered to express one of several versions of a fluorescent protein derived from jellyfish. These enhancements add to the commercial value of the pet fish. Experiments show that the same enhancements would make the fish more vulnerable to predation if they were to be accidentally released into the wild. Soon, society will be forced to grapple with the implications of human genetic enhancement for nonmedical, non-research purposes, such as sports performance enhancement. For example such activities may be undertaken by "rogue" operators, working outside the jurisdiction of normal regulatory regimes. Because human subjects research for these purposes is not currently permissible, IRBs and IBCs are not expected to face these questions in the near future, although they remain an outstanding challenge for society at large.

disease). Because untreated SCIDS is inevitably fatal, it is easy to recognize SCIDS as a disease, worthy of therapeutic intervention.

The determination of what constitutes a genetic disease or defect is not always so simple. Unlike SCIDS, the degree to which most of our genetically-determined traits are perceived as "good" or "bad" is culturally defined, and those cultural perceptions can be colored by racial, political, and economic factors. Therefore is it important to recognize the difference between interventions that are *therapeutic*—intended to treat a medically-recognized disease, versus those that are *enhancement*—intended to alter inherited traits that are not involved in a diagnosable disease state. *Gene therapy* is currently considered a legitimate application in human subjects research; *genetic enhancement* is not. Genetic enhancement of human subjects is not currently permitted anywhere. (Genetic enhancement of nonhuman animals for scientific and commercial purposes is a current and controversial fact of life.)

As discussed above, another consideration in human gene therapy is whether the intervention is designed to affect only a targeted individual, or to affect future generations. All current gene transfer applications involving recombinant DNA and currently contemplated for testing in human beings are designed to affect only an individual person. Indeed, current approaches are designed to affect only a tiny fraction of the total cells in the subject's body. It is also possible to design genetic interventions that would affect the DNA of germ line cells. Speculatively, germ line genetic modification for

both therapeutic and enhancement purposes has been contemplated, but human subjects research involving recombinant germ line modification is not permitted anywhere.

It is worth noting that intentional manipulation of the DNA content of germ line cells in human subjects is already occurring and was recently approved as a medical intervention in the UK. *Mitochondrial diseases* are caused by variations in the mitochondrial DNA that is naturally inherited from the mother. In order to prevent passing mitochondrial diseases on to their children, some affected women have chosen to participate in *mitochondrial replacement therapy*, whereby mitochondrial DNA from a healthy individual (the so-called "third parent") is transferred during the process of artificial fertilization, such that the resulting zygote has mitochondrial DNA lacking the disease-associated variant. Female children resulting from this procedure will pass on the artificially-transferred genetic information to their own children and to subsequent generations indefinitely. Because such interventions use unmodified DNA taken directly from the third parent donor's cells, rather than recombinant DNA, the procedure is perceived as less dangerous and controversial.

One form of genetic engineering that has received intense public attention in recent years is "CRISPR" (Clustered regularly interspaced short palindromic repeats)-based genome editing. It is important to note that the basic ethical and regulatory questions related to CRISPR-based technologies are not fundamentally

CRISPR Technologies

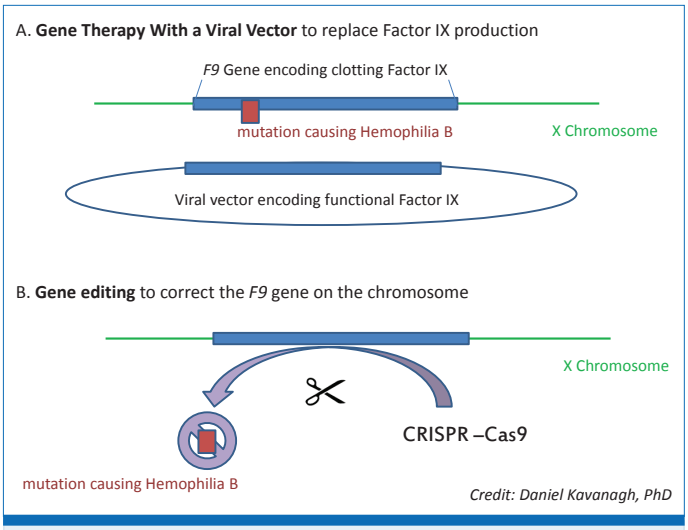
As with almost every important biotechnology invention, CRISPR (Clustered regularly interspaced short palindromic repeats)-based technology has its origins in basic science investigations of microbiology. In the late 1980s, repeated clustered DNA sequences were observed in the genomes of several bacterial species. In the 2000s, it was found that these sequences corresponded to DNA sequences of viruses that attack bacteria-- the CRISPR sequences were a tool used by bacteria to recognize viral DNA and steer "scissors" proteins, called "Cas" proteins to cut the viral DNA.

In 2012 Jennifer Doudna and Emmanuelle Charpentier at UC Berkeley demonstrated that a genetically-engineered CRISPR-Cas system could be used to "edit" any DNA sequence chosen by the investigator. In 2014, the groups of Feng Zhang and George Church at MIT and Harvard demonstrated that this approach could be applied to edit genes in living mammalian cells. As the commercial potential of CRISPR-Cas technologies became apparent , the overlapping discoveries of the Berkeley group and the MIT groups have led to a prolonged patent fight relating to technologies that may eventually produce billions of dollars of revenue. In early 2017, a patent court found that the work of Zhang's group was non-obvious enough that their patents did not constitute "interference" with the prior patents filed by Doudna and Charpentier—meaning that Zhang and MIT have a very strong patent position with respect to commercialization of CRISPR-Cas technology moving forward .

different from those associated with older techniques. CRISPR-based techniques potentially allow more precise and more efficient DNA modification than older technologies. This certainly makes it more practical to consider bringing new applications to the clinic, and that in turn makes it more important for us to consider the practical and ethical implications of genome editing today. CRISPR technology is already being used in non-germline immunotherapy clinical trials currently underway.

With the advent of CRISPR, an associated technical approach, the *gene drive*, has also come to public attention. A gene drive is a technical approach where the molecular tools for gene editing are encoded into the DNA of the target organism. A gene drive is designed to alter the course of evolution of a breeding population. Gene drives have been proposed as a means to alter or eliminate wild populations of pest species such as mosquitoes. It has been proposed that self-inactivating gene drive technology might someday be ethically-permissible in humans, but such applications are currently speculative and untested. Gene drives are mentioned here only to avoid confusion with other, ethically plausible, applications of CRISPR technology.

In conclusion, gene therapy research, when intended to treat diseases in individual subjects, is fundamentally similar to experimental therapies involving chemical compounds or biologicals. The potential application of genetic modification for the purposes of enhancement, or for the alteration of germ line cells to affect future generations, creates new opportunities and challenges for physicians, ethicists, and the general public.



Vector-Mediated Gene Therapy vs. Gene Editing

Hemophilia B is caused by a mutation in the *F9* gene, encoding clotting Factor IX, on the X chromosome. If a boy inherits an X chromosome with a particular defect (mutation) in the DNA sequence of the *F9* gene, he will suffer from Hemophilia B due to an inability to produce functional Factor IX. Two genetic approaches to treat Hemophilia B are shown. **A. Gene Therapy With a Viral Vector:** recombinant DNA encoding a functional *F9* gene is introduced into some of the subject's cells (for example, liver cells). The gene transfer leads to production of functional Factor IX without altering the DNA sequence of the chromosome. **B. Gene editing:** CRISPR-Cas9 system is used to edit the DNA on the chromosome—the mutation is corrected *in situ* and the resulting "edited" chromosome encodes fully functional clotting factor.

About the Author

Daniel Kavanagh, PhD, is Senior Director of Biosafety and Gene Therapy at WCG Biosafety, a division of the WIRB-Copernicus Group. Prior to joining WCG, he was Assistant Professor of Medicine at Harvard Medical School, where he was Vice Chair of an Institutional Biosafety Committee, and co-chair of a Phase 1 human gene transfer vaccine trial in HIV+ subjects. Dr. Kavanagh holds a PhD in Molecular Microbiology and Immunology from the Oregon Health and Science University, and did postdoctoral training at Harvard Medical School, and Rockefeller University.

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