Human Genome Editing FAQ

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DEFINITIONS

What is a genome?

The genome is the instruction book of a cell that is encoded by four letters found in DNA (A, G, T & C, also known as bases). The order, or sequence, in which these letters are written are translated by cells ultimately leading to the manufacture of proteins which enable cells to grow, survive & communicate with other cells. The genome can be broadly broken into two components 1) coding DNA called genes that encode the proteins and 2) non-coding DNA which regulates when and how genes are turned on within the cell. Within humans there are approximately 20,000 genes that encode the human body. Of those 20,000 genes there are more than 6,000 genetically based diseases where the DNA sequence has been changed in such a way as to result in abnormal protein levels or function.

What is genome editing and how does it work?

Genome editing is a process by which a DNA sequence is modified to elicit a desired outcome within a living cell**.** Though DNA modification techniques have existed for decades, recent advances in genome editing technology have provided scientists and researchers with far more precise and efficient genome editing tools. Scientists are exploring myriad potential uses for these tools, including agricultural, environmental, and clinical applications. In basic research, gene editing is being used to determine the roles different genes play in disease. Medical researchers are exploring ways to use genome editing to treat or prevent genetically-defined human diseases.

Genome editing is based on a naturally occurring system that directs a molecular scissors, called a nuclease, to a target region of DNA. The DNA is targeted by a recognition signal that is specific to a fragment of DNA. Once the nuclease has been directed to the appropriate region of DNA it cuts the DNA. The DNA is then repaired by a process found naturally in every one of our cells. This repair machinery is there to repair any break that may occur within your DNA.

Once the DNA is cut, the cell can be directed to repair that region of DNA in three different ways:

1) Insertion of a DNA sequence, when a template DNA is provided to the cell in parallel with the targeted cutting.
2) Deletion of a DNA sequence, when two regions of DNA are cut
3) Change of a DNA sequence, when a corrective template DNA is provided

What is the difference between somatic cell and germline genome-editing?

Somatic cells are cells whose genetic material *cannot be passed on to future generations of people*. The vast majority of cells in the human body are somatic cells, examples of which include the tissues that make up our skin, muscles, lungs, liver, and heart as well as blood cells. Changes to somatic cells will only impact an individual patient who consents to participate in a genome editing procedure. Current therapeutic applications of genome editing focus on somatic cells.

Germline cells (or germ cells) are any cells in multicellular organisms whose genetic information may be inherited by future generations of that species. In humans, examples of germ cells include sperm or egg cells, fertilized embryos, or reproductive stem cells. As such, any genetic manipulations in germ cells could be passed down to future generations if

they resulted in a pregnancy that came to term. Such changes would then be introduced into the human gene pool, or total collection of heritable human genetic information.

How many kinds of genome editing technologies exist, and how do they differ (TALENs, Zinc Fingers, and CRISPR)?

Broadly speaking, there are three major genome editing technologies in use today to make targeted DNA edits. Although each works slightly different, all of them rely on nucleases—proteins that cut DNA—and they can all bind to and edit targeted genes. These include zinc finger nucleases (**ZFNs**), transcription activator-like effector nucleases (**TALENs**), and the more recently discovered Clustered Regularly Interspaced Short Palindromic Repeats (**CRISPR)** nucleases. **Meganucleases** represent a fourth category, but are not as widely used for potential clinical applications. The decreased cost and increased speed of making specific DNA and RNA has greatly improved the efficiency of these technologies.

All gene editing technologies can be used to delete, insert, or repair the sequence of DNA with the use of a nuclease enzyme. Once optimized for cell type, these nucleases can work on any type of DNA including that of humans, animals, plants, or microorganisms like bacteria.To insert or make corrections in the DNA, a new template DNA must be added to the therapy along with the nuclease. All gene editing systems must also be delivered into the body as DNA or RNA in order to function.

Zinc Finger and TALEN platforms rely on modular proteins, in addition to the nuclease, to recognize a target sequence of DNA (e.g. a gene). Modification of the Zinc Finger or TALE proteins allows for a variety of DNA sequences to be targeted for editing. Due to the optimization process of the binding proteins, these platforms allow for high on-target efficiency.

The CRISPR genome editing system utilizes specifically designed guide RNA that can find and bind to a specific DNA sequence. The CRISPR nuclease then edits or removes the targeted DNA that is the root cause of a disease. Different guide RNAs can be created to target different DNA sequences enabling multiple sites within a genome to be targeted in a single procedure. This provides for the versatility to address complex diseases at the genetic level.

SOMATIC CELL GENOME EDITING IN HUMANS TO TREAT DISEASE

What are the potential applications and benefits of human genome editing technologies to treat or cure disease?

Genome editing technologies hold tremendous promise to treat genetically-defined diseases. Using genome editing, scientists and clinicians may not only only treat the symptoms of a disease, but address its underlying root cause at the genetic level. Genome editing has the potential to someday mitigate, prevent, or cure genetically defined diseases. Depending on the disease and treatment in question, researchers expect that some genome editing treatments might need to be repeated, whereas others may only need to be given once. In some cases, genome editing may be used to create healthy edited cells that divide, crowd out, and replace their unhealthy counterparts. It is hoped that these last cases lead to a permanent benefit.

Research is currently underway on clinical applications of genome editing technologies to treat genetic disorders like sickle cell disease, cystic fibrosis, congenital blindness, hemophilia, amyloidosis, and lysosomal storage disorders. In addition, significant progress in therapetutic genome editing has been demonstrated in cancer and infectious diseases, such as HIV and Hepatitis B. (See page 6 for examples of human genome editing to treat/cure disease.)

How do current genome editing technologies differ from other genomic medicines (*e.g.* gene therapy)?

Genomic medicine is a broad term that describes any medical intervention that uses knowledge of genetics to guide the care of patients and the development of new therapies. The goal of genome editing is to target and alter disease-affiliated genes at the DNA level to cure, mitigate, or potentially prevent disease. It can be contrasted with a second major type of genomic medicine: gene therapy.

Gene therapy refers to a method that introduces one or more new copies of a gene into the patient's genome to restore cell function despite the continued presence of the mutated gene. In order to have a durable effect, the new genes must be expressed for a prolonged period of time (ideally, the entire life of the patient).

Genome editing, by contrast, corrects or removes a defect in the natural context of that gene. Once changed, the correction will persist throughout the life span of the cells, or be faithfully passed to all decedents of the originally edited cell in an individual patient. From a dosing perspective, it is anticipated that a cell in a patient would only need to be exposed to genome editing therapy for a short duration of time in order to achieve the desired genetic change.

How does somatic cell genome editing work in patients?

Somatic cell genome editing can happen **outside the body (*ex vivo*)** or **inside the body (*in vivo*).** Each method has benefits and limitations, and preference of method depends on the disease being treated.

In ***ex vivo* genome editing**, the damaged target cells—for example, blood cells—are first removed from the patient. The cells are then isoloated and sustained in a laboratory before undergoing genome editing treatment to target and edit (or fix) the damaged gene. Upon successful editing, the 'fixed' normally functioning cells are returned back to the patient. *Ex vivo* editing allows researchers to examine the accuracy of the editing in the laboratory before the cells are returned to the patient, but ex vivo editing can only be performed in cells that can be safely removed from that patient, kept alive in a laboratory, and then given back to the patient in exactly the right location in the body. This is why *ex vivo* genome editing is being tested mainly for blood and immune disorders, both of which involve cells from the bloodstream or bone marrow that can be collected, grown, and edited outside the body, and then returned to the patient.

***In vivo* genome editing** occurs inside a human body and therefore can potentially address many more diseases than the *ex vivo* process. During *in vivo* genome editing, the editing therapeutics are delivered directly to the target site by using a vector. The delivery vector can either be a virus that is naturally benign or altered so that it is rendered completely harmless to a patient, or a chemical-based, non-viral cargo that carries the editing therapy. Other non-viral vectors are also being explored. The vector travels through the body to find and enter the target cells where it can deliver the editing therapy.

In some cases, genome editing can be used to create healthy cells with corrected or edited genes that will divide and reproduce inside the body until they crowd out and replace the unhealthy cells containing the unedited (flawed) gene, thus leading to a permanent benefit. Depending on the type of cell involved in the treatment and the lifespan of these cells in the body, researchers expect that some genome editing treatments might need to be repeated to achieve therapeutic benefits, whereas others might only need to be given once.

How does a patient get treated with somatic cell genome-editing medicines?

The actual items that patients will be treated with will depend on whether genome editing needs to be performed *in vivo* or *ex vivo* (see above). Hypothetically, after cells are edited *ex* vivo a patient would need to have their own edited cells readministered to them by a procedure like intravenous infusion. To perform *in vivo* editing a patient needs genome editing therapy delivered to the target tissue, so an *in vivo* genome editing medicine may look like a direct injection or an inhalation.

Are genome editing technologies currently being studied in human clinical trials?

Yes. The first zinc finger based somatic cell genome editing treatment to enter U.S. clinical trials in humans targets was completed and published in early 2014. This 12 patient study used *ex vivo* gene editing to knockout (or disrupt) a gene in immune cells of patients with HIV enabling them to fight off infection. A number of other human clinical trials are expected to begin in the U.S. in 2017. Genome editing trials using CRISPR technology have also recently begun in China studying an ex vivo gene disruption approach to treat lung cancer. (See page 6 for examples of human genome editing to treat/cure disease)

How far away are we from having an approved human genome editing medicine?

We are still some years away from having genome editing medicines approved by the FDA. At present, there are currently a number of clinical trials under way involving human genome editing technologies, which are designed to assess the safety and effectiveness of these therapeutic approaches. (See page 6 for examples of human genome editing to treat/cure disease.)

What is the current state of the science in genome editing in humans to treat or cure disease?

There is a tremendous amount of basic research currently underway with genome editing tools. Gene editing technologies have allowed for the rapid development of cell and animal models of disease. The specificity of these platforms, allows scientists to uncover the roles certain genes play in disease. The drop in DNA sequencing costs, synthesis costs, and the development of DNA delivery methods have expedited the development of the first therapeutics.

All preclinical and clinical testing by BIO member companies using genome editing for the treatment of disease are being delivered into **somatic** cells, either *in vivo* or *ex vivo*.

Preclinical animal research using gene editing therapies has been underway since 2008, when the first *ex vivo* Zinc Finger Nuclease (ZFN) experiment was completed. The first somatic cell *in vivo* preclinical studies were reported in 2011. Scientists have made advances in the translation of these preclinical efforts into potential human therapies.

Human clinical trial research efforts for somatic cell human genome editing technologies (*ex vivo* and *in vivo)* are scheduled to being in early 2017. These editing techniques offer the potential to provide lifelong or curative treatments for human diseases. In addition to increasing the percentage of correctly edited target cells for each therapy, these early trials aim to minimize 'off-target' edits and possible immune responses. Researchers advancing the science of *in vivo* approaches are also working to address potential complications associated with delivery, i.e. making sure the genome editing occurs only on the specified target and does not cause a harmful immune response or other undesirable effects on the patient.

It is critical to understand that there are regulatory frameworks that carefully evaluate human genome editing in clinical trials before they start and while they are underway. (See page 8 on regulatory oversight for more information.)

Because of these projects, the scientific community's understanding of genome editing technologies is evolving daily. And as the scientific community's knowledge advances, the potential benefits to patients increases and the risks to patients decreases.

Examples of human genome editing to treat/cure disease in human clinical trials or being researched

HIV

HIV (human immunodeficiency virus) infection of the body's immune system can lead to the disease AIDS (acquired immunodeficiency syndrome).

The first Zinc Finger Nuclease (ZFN) based somatic cell genome editing treatment to enter U.S. clinical trials in humans was published in early 2014.[1] This 12 patient study used *ex vivo gene editing* to knockout (or disrupt) a gene in immune cells of patients with HIV. (See page 3 for information on ZFN based genome editing.)

Leukemia

Another area where genome editing is currently undergoing investigation is in the treatment of certain forms of cancer. The goal is to edit genes within human immune cells—similar to how these cells are edited to treat HIV (see above)—to increase their ability to recognize and attack cancer cells. This is accomplished by modifying T-cells (immune system cells) so they can identify and attack specific cancer cells.

In November 2014 a one-year-old girl with leukemia was successfully treated using donor CAR T cells that were edited *ex vivo* using TALEN genome editing. In early 2017, two girls were reported to be in full remission using this technology. (See page 3 for more information on TALEN genome editing.)

[1] Tebas, P, et al. *Gene Editing of CCR5 in Autologous CD4 T Cells Infected with HIV.* N Engl J Med 2014; 370:901-910. March 6, 2014. DOI: 10.1056/NEJMoa1300662. Available at: http://www.nejm.org/doi/full/10.1056/NEJMoa1300662

Hemophilia

Hemophilia is a heritable genetic disorder that results in deficiencies in proteins responsible for forming blood clots that leave patients at higher risks of complications from hemorrhaging. Presently there are no curative therapies for Hemophilia diseases, only chronic treatments meant to mitigate symptoms.

As of February 2017, a Zinc Finger Nuclease (ZFN) *in vivo* liver cell genome editing program for Hemophilia B was in phase 1/2 clinical trials. Hemophilia A ZFN programs are currently in preclinical stages. (See page 3 for information on ZFN based genome editing.)

Leber Congenital Amaurosis 10

Leber Congenital Amaurosis (LCA) refers to a set of genetically inherited diseases caused by mutations that eventually lead to blindness. More than 18 of these mutations are known to cause the degeneration of cells called photoreceptors—cells that absorb light and enable us to see. These photoreceptor cells steadily die off over time, leading to blindness. The aim of genome editing interventions is to deliver editing components to target cells to facilitate precise editing of these mutations.

Clinical trials to treat LCA10 using CRISPR genome editing could begin as early as 2017. (See page 3 for information on CRISPR based genome editing.)

Mucopolysaccharidosis

Mucopolysaccharidosis Type I (MPS I) and MPS II are inherited metabolic disorders known as lysosomal storage disorders (LSDs). They are caused by mutations in genes encoding enzymes that break down unwanted substances in cells. The resulting cell damage can lead to serious health problems.

In 2017, Phase 1/2 clinical trials are expected to begin for MPS I and MPS II using ZFN genome editing. The approach is designed to enable the liver to permanently produce circulating therapeutic levels of a corrective enzyme product. Ultimately, the target population for this approach will include pediatric patients for whom it is important to be able to produce stable levels of therapeutic protein for their lifetime. (See page 3 for information on ZFN based genome editing.)

Sickle Cell Disease

Sickle Cell Disease is a common genetic disorder that affects red blood cells' ability to deliver oxygen to our bodies. It is caused by a single gene for the protein responsible for carrying oxygen. This mutation ultimately causes red blood cells to change from their normal round shape into a crescent moon—a change that in turn makes cells clump together within small blood vessels. This prevents normal oxygen delivery, causes internal damage to tissues and organs, and causes patients to experience severe pain and a lifespan shortened by several decades.

One approach to treat sickle cell disease using genome editing attempts to correct the mutation in blood cells *ex vivo* before returning them to the patients' bone marrow so they can produce healthy red blood cells. Promising preclinical work is being demonstrated in animals using both AFN and CRISPR platforms. (See page 3 for information on ZFN and CRISPR based genome editing.)

Amyloidosis of the Transthyretin (ATTR)

Transthyretin amyloidosis (ATTR) is a condition resulting from a genetic mutation that can lead to severe loss of nerve or cardiac function due to accumulation of transthyretin protein in tissues.

A preclinical study targeting TTR showed that over 60% of the target gene could be knocked out with a single dose of CRISPR through a non-viral delivery system called a lipid nanoparticle**.** (See page 3 for information on CRISPR based genome editing.)

Cystic Fibrosis

Cystic Fibrosis is a progressive, genetic disease that causes persistent lung infections and limits the ability to breathe over time. In people with Cystic Fibrosis, mutations in the CFTR gene cause a thick, buildup of mucus in the lungs, pancreas and other organs. In the lungs, the mucus clogs the airways and traps bacteria leading to infections, extensive lung damage and eventually, respiratory failure. In the pancreas, the mucus prevents the release of digestive enzymes that allow the body to break down food and absorb vital nutrients.

Preclinical research is currently underway to use the CRISPR system to repair the CTFR gene in patients with cystic fibrosis. (See page 3 for information on CRISPR based genome editing.)

SOMATIC CELL HUMAN GENOME EDITING MEDICINES - OVERSIGHT AND REGULATORY FRAMEWORKS FOR APPROVAL

What oversight and regulatory frameworks currently govern approval of human genome editing medicines in the U.S.?

Over the past 40 years, the United States has continuously added to a biomedical R&D framework of laws, regulations, and guidelines to keep pace with advances in genomics. Today, research in genomic medicines is principally governed by the U.S. Department of Health and Human Services (**HHS**) and two of its constituent agencies: the Food and Drug Administration (**FDA**) and the National Institutes of Health (**NIH**).

In addition, any research that requires human volunteers as research subjects must be regulated by local safety and ethics review committees. These local review committees are managed by the HHS Office of Human Research Protections (**OHRP**).

Current regulatory language in the United States covers genome editing through its references to "genetic therapies" or "genetic manipulations." The intention of gene therapy and genome editing clinical applications in somatic cells is so analogous that the policies in place effectively govern both.

Dr. Robert Califf, former FDA commissioner, clarified on January 18th, 2017 that "*Human medical products* that apply gene editing to exert their therapeutic effect are regulated under our existing framework for biological products, which include gene therapy products" and that "FDA's Center for Biologics Evaluation and Research (**CBER**) has a well-established program and policies in place to evaluate gene therapy products."

A 2017 U.S. National Academies of Science (**NAS**) report on human genome editing concluded that "clinical trials of genome editing in somatic cells for the treatment or prevention of disease or disability should continue, subject to the ethical norms and regulatory frameworks that have been developed for existing somatic gene therapy research," a view shared by the American Society of Gene and Cell Therapies (ASGCT).

Any genome editing research conducted with federal funding or that occurs at an institution that accepts federal funds is overseen by the NIH and is likely to be reviewed by its recombinant DNA advisory committee (**RAC**). Any research that aims to use or create medical products is regulated by the FDA. All human cells that are genetically manipulated outside of the body are considered medical products and are regulated by the FDA, including reproductive cells or embryos. This covers all *ex vivo* editing of human cells for clinical aims. Any human cell genome editing that would occur *in vivo* requires the administration of a genome editing drug falls under FDA jurisdiction.

The established US regulatory frameworks covering genetic therapies have recently been used to review the initiation of gene editing clinical trials. Zinc Finger Nuclease clinical trials were reviewed and initiated back in 2011. More recently, CRISPR clinical trials have been reviewed. FDA Commissioner Robert Califf commented in early 2017, "The RAC [the NIH's Recombinant DNA Advisory Committee] recently discussed (and did not find any objections to) the first clinical protocol to use CRISPR/Cas9-mediated gene editing".

What oversight and regulatory frameworks currently govern approval of human genome editing medicines internationally?

Each country maintains its own regulatory frameworks governing the application of human genome editing technologies and other genetic manipulations in their own countries. In Europe, the European Medicines Agency utilizes a Committee for Advanced Therapies to assess quality, safety, and efficacy of new technologies for biologic medicines.

GERMLINE HUMAN GENOME EDITING - REGULATORY OVERSIGHT & CONSENSUS ON ETHICAL STANDARDS

How is germline human genome editing regulated in the U.S.?

FDA considers all *ex vivo* genome edited cells to be medical products, including reproductive cells and IVF embryos. A 2016 federal funding bill--Section 749 the Consolidated Appropriations Act of 2016--states that the FDA cannot evaluate research or clinical applications that modify gametes, embryos, or germline cells to cause heritable genetic modifications. This effectively precludes the possibility of any germline clinical research applications, both *ex vivo* and *in vivo*.

NIH does not support germline genome editing research in human materials. Appendix M of the *NIH Guidelines* explicitly states that the recombinant DNA advisory committee (RAC) will not accept clinical research protocols involving germ-line modification or in utero gene transfer. In addition, the Dickey-Wicker Amendment (DWA), passed in 1996, added a set of specific restrictions on the NIH's ability to support embryo research. The DWA forbade the NIH from using federal funds to create embryos for research purposes, or to fund research in which embryos are destroyed, discarded, or damaged. The DWA is still active today, and prevents federal research funding from being used to study human embryo genome editing.

While all of these measures prevent the possibility of clinical applications or federally funded basic research in human germline editing, privately funded basic research remains legal in the United States.

How is germline human genome editing regulated internationally?

A 2014 review of international policies on germline alterations found that, of 39 countries surveyed, 29 of these had bans in place to prevent germline editing. Twenty-five of these countries had bans based in legislation (Canada, Mexico, Brazil, the EU, Israel, Australia, New Zealand) while four countries' bans were based in less enforceable research guidelines (China, Japan, India, Ireland). Excluding the US, the remaining nine countries had ambiguous policies (Russia, Iceland, South Africa, Peru, and Chile).[2]

While the European Union (EU) ostensibly bans germline modifications, the EU is also complex. The general EU agreement in opposition to germline editing was first solicited by the **Oviedo Convention** Agreement of 1997, which unequivocally opposes human germline modification but permits human somatic alterations only for preventative, diagnostic, or therapeutic reasons. Though many member states have signed the agreement, not all signing states have ratified its measures, leaving non-ratifying nations to design their own regulations on human germline editing.

What is the consensus on genomic manipulation procedures that would affect human germline cells?

For decades, the academic and industrial research communities have observed a ***voluntary moratorium on genomic manipulation procedures that would affect human germline cells.*** In addition, a number of regulatory measures exist in the U.S. to preclude the possibility of human germline manipulations (See page 2 for more information).

One conclusion reached by the U.S. National Academies of Science (**NAS**) in its 2017 report, is that "there is a need for caution in any move toward germline editing, but that caution does not mean prohibition." It recommended that under a very strict set of 10 criteria, when no other reasonable alternatives exist, government restrictions have expired, and research on risk/benefit standards has advanced in the field, that certain germline editing could be permissible for the treatment of disease.

[2] Araki, M., & Ishii, T. (2014, November 24). International regulatory landscape and integration of corrective genome editing into in vitro fertilization. *Reprod Biol Endocrinol Reproductive Biology and Endocrinology, 12*(1), 108. doi:10.1186/1477-7827-12-108

What is the consensus on permitting genomic editing of embryos for basic research? For clinical application?

This issue has been addressed on a country-by-country basis. The ***United States government does not fund any research*** involving the editing of human embryos, neither will it review nor approve any clinical applications of genome editing technologies that result in the genetic modification of a human embryo.

BIO acknowledges this policy and notes that BIO's member companies are focused on therapeutic applications of genome editing of ***somatic cells*** to cure, mitigate, or potentially prevent disease.