

CRISPR UPDATE: CONSIDERATIONS FOR A RAPIDLY EVOLVING AND TRANSFORMATIVE TECHNOLOGY

MICHELLE KIRTLEY, PHD

CBHD BIOETHICS & PUBLIC POLICY ASSOCIATE

Earlier this year, only a few months after the Chinese stunned the world by announcing that they had edited genes in human embryos,¹ the British Human Fertilisation & Embryology Authority (HFEA) gave researchers in the U.K. permission to conduct similar experiments with human embryos who have been abandoned at local fertility clinics.² This research—made possible by a technological revolution in DNA editing called CRISPR/Cas9—is controversial not only because of the moral status of the embryos involved but also because of the potential for permanent changes to be introduced into the human gene pool by means of germline intervention, and fears of the further commodification of human life in the form of designer babies.

For decades, researchers have been introducing changes into the DNA of model organisms in order to better understand the function of those particular stretches of DNA and the role different genes play in the progression of disease. Some scientists have tried to use similar techniques in humans to correct diseases known to be caused by a single DNA mutation, but gene therapy based on these older technologies has proven extremely difficult to use safely in human patients. In fact, after almost 25 years of research and development, the Food and Drug Administration (FDA) has yet to approve any gene therapy product for sale in the U.S. These older gene editing technologies are also labor intensive, expensive, and inefficient.

The new CRISPR/Cas9 system, an adaptation of an elegant, naturally occurring gene splicing mechanism, offers several advantages over its predecessors. The CRISPR/Cas9 system was originally identified as a tool that bacteria use for defending themselves against infection caused by foreign DNA found in small, virus-like pathogens. The system contains a “homing mechanism” which is able to locate and bind to a very specific sequence of DNA and a pair of “molecular scissors” which cut the target DNA at a precise location within that sequence. Scientists have transformed this naturally occurring tool into a “find and replace” system that can edit DNA sequences efficiently and specifically. Depending on whether the goal is to obliterate gene function or introduce specific changes in the

DNA sequence, different modifications of the CRISPR/Cas9 system are used. The system has worked in almost every organism tested, including organisms previously resistant to more traditional forms of DNA manipulation.

Transformative Technology with Far-Reaching Applications

The applications of this technological revolution are profound. Yogurt producers are interested in using the system as originally designed, to protect their bacteria from infections that can ruin large batches of yogurt.³ Several agribusinesses are interested in using CRISPR/Cas9 to create genetically modified livestock and crops.⁴ In 2015, Cibus became the first company to bring a genome-edited product (herbicide-resistant Canola) to the market in the U.S.⁵ These products are distinct from GMO (genetically modified organisms) food because CRISPR-modified organisms do not contain foreign DNA or “transgenes.” The U.S. Department of Agriculture (USDA) has already ruled that organisms modified with prior generation editing techniques do not require special approval (as traditional GMO agriculture does) because they do not involve the use of “plant pests” to introduce changes to the plant’s DNA.⁶

As a laboratory tool, CRISPR/Cas9 is opening new ways for scientists to model human disease and develop potential treatment options. In 2014, scientists used CRISPR to precisely target two genes in cynomolgus monkeys (a variety of macaque), the first time researchers were able to selectively disrupt genes in primates.⁷ Scientists have shown that a mutation associated with tyrosinemia, a human metabolic disease, could be corrected in an adult mouse using CRISPR/Cas9 to “fix” the mutation.⁸

Some researchers are exploring the use of CRISPR/Cas9 to create gene-drives. With the use of this technology, scientists could eradicate vector-borne diseases such as yellow fever, malaria, or Zika by engineering disease-free mosquitoes specially designed to take over the entire mosquito population in a few generations.⁹ While the elimination of these diseases could have an enormous public health benefit, critics have urged caution, since the release of these organisms could have unintended ecological consequences, as there is no way to control the genetic

drift of the engineered mosquitoes once released into the wild.

One of the key reasons that the CRISPR/Cas9 system has generated so much enthusiasm is its potential for use in human gene therapy protocols. CRISPR/Cas9 could be used to correct mutations in human adult stem cells or induced pluripotent stem (iPS) cells. These edited cells could then be transplanted back into the patient to treat diseases. In basic laboratory experiments, scientists have already used CRISPR/Cas9 to excise HIV from the DNA of human cells and to correct a mutation that causes a blood disorder called Fanconi’s anemia in iPS cells that are then differentiated into hematopoietic (blood) stem cells.¹⁰ Although this has not yet been tested in human patients, these now-healthy stem cells could in theory be transplanted back into a human patient to reconstitute a healthy blood cell population. Researchers are exploring a similar technique to re-engineer patients’ blood cells to become HIV-resistant.¹¹

4 Germline Intervention & Potential Implications

Although much work remains to translate these promising results into safe and effective human therapies, these techniques have not generated ethical controversy because they manipulate the DNA of somatic cells rather than germline cells or embryos. Somatic cells include all of the cells in our bodies that are not involved in reproduction. Genetically modifying somatic cells will not affect the human gene pool because the edited DNA cannot be passed onto the patient’s children.

The opposite is true of germline cells—the egg and sperm cells that become future human beings. Changes made to these cells (or to embryos created in vitro) will be passed to almost every cell of the next generation and can be inherited by future generations. Since the early days of modern genetic engineering, when researchers first discovered how to cut and splice DNA, researchers maintained that permanently altering the

human gene pool was a bright line that should not be crossed.¹² The American Medical Association, for example, currently maintains that, “The fundamental difference between germ line therapy and somatic cell therapy is that germ line therapy affects the welfare of subsequent generations and may be associated

“*Since the early days of modern genetic engineering, when researchers first discovered how to cut and splice DNA, researchers maintained that permanently altering the human gene pool was a bright line that should not be crossed.*”

with increased risk and the potential for unpredictable and irreversible results. Because of the far-reaching implications of germ line therapy, it is appropriate to limit genetic intervention to somatic cells at this time.”¹³ But this opinion was written in 1996, before modern DNA editing technologies were on the horizon. Now what was previously unimaginable—and therefore easy to oppose—is now possible.

In April of 2015, Chinese researchers reported that they had “successfully” used CRISPR/Cas9 to edit a mutation known to cause β -thalassemia (a serious blood disease) in human IVF embryos.¹⁴ Although only 4 of the 86 embryos that were injected with the CRISPR-Cas9 system were shown to contain the corrected DNA sequence, this study demonstrated the technique’s feasibility. Now, scientists can tweak and refine the technique in the pursuit of therapies for previously intractable diseases and to understand the very first steps in human development.

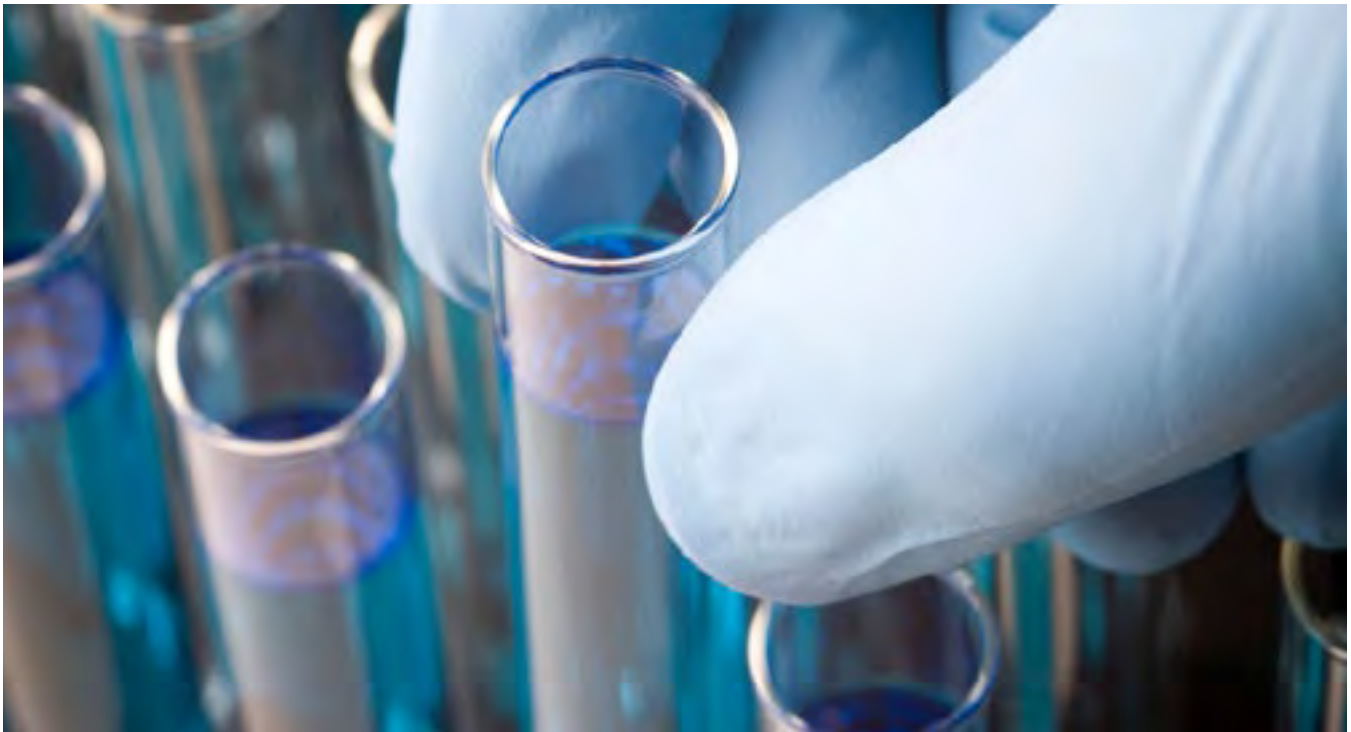
While most voiced some level of concern, the reaction of the scientific community was mixed. Harvard Professor David Sinclair told *Technology Review* that “[p]eople would look back at this moment in time and recognize it as a new chapter in ‘how humans control their bodies’... because it would let parents determine ‘when and how they have children and how healthy those children are actually going to be.’”¹⁵ Edward Lanphier, biotech

CEO and Chairman of the Alliance for Regenerative Medicine, voiced a different perspective, saying, “Many oppose germline modification on the grounds that permitting even unambiguously therapeutic interventions could start us down a path towards non-therapeutic genetic enhancement. We share these con-

cerns.”¹⁶ And CRISPR pioneer Jennifer Doudna said, “It cuts to the core of who we are as people, and it makes you ask if humans should be exercising that kind of power.”¹⁷ This ambivalence among key figures in the biomedical research community is notable since they each support the use of discarded IVF embryos for the purpose of embryonic stem cell research.

Knowing that the Chinese results were forthcoming, Doudna and other prominent scientists and bioethicists convened a meeting to discuss what the collective response of the scientific community in the U.S. should be. Many of those present signed a document calling for a moratorium on the creation of genetically modified children but endorsing research on human embryos, reminiscent of a similar agreement forged when recombinant DNA technology first emerged.¹⁸ Uncomfortable with the prospect of designer babies, these researchers nonetheless are interested in the potential of CRISPR/Cas9 to cure genetic diseases and unravel early human development.

Like the U.S., the U.K. had a similar and even more binding moratorium on germline genetic engineering. Recently, however, the HFEA reversed course and granted a license for a team of scientists to use CRISPR/Cas9 to genetically modify healthy human embryos discarded from fertility clinics under the condition that these embryos be destroyed and never implanted into a woman’s uterus.¹⁹ Kathy



Niakan and her team want to modify genes involved in the earliest stages of human development to learn exactly how these complex processes are regulated in the hopes of better understanding the causes of infertility.

Ethical Concerns for Germline Interventions

As excitement and momentum about the promise of germline genetic engineering builds, we must of course pause and ask: is it prudent, and is it ethical? The short answer is no. In addition to the serious safety concerns raised by germline genetic modification, there are several arguments against the use of CRISPR/Cas9 in human embryos and human germline cells.

First, germline genetic engineering violates the autonomy of future generations because it is impossible to obtain their consent for the genetic manipulation they will inherit. Bioethicists from widely divergent ethical and philosophical traditions have agreed on the importance of informed consent in human biomedical research. This principle guards against the commodification of other human beings in the quest for scientific progress.

Demanding that future generations serve our ends—however noble they may be—crosses this line.

Second, although our methods of manipulating and sequencing DNA have progressed rapidly, our understanding of exactly how genotype (the precise DNA sequence of a gene) relates to phenotype (the characteristics that we can observe or measure) remains primitive. When sequence variations are observed, scientists struggle to determine which are simply part of “normal” variation within

that interact with DNA. In recent years, what was thought to be “junk” DNA is now known to play a role when certain genes are active or inactive. Gleaning meaningful information from the massive amounts of DNA sequence data that have been collected requires sophisticated algorithms that push the limits of current computing power. Furthermore, our ability to meaningfully sort out sequence data is limited by the fact that our databases of DNA sequences lack ethnic diversity.

“As excitement and momentum about the promise of germline genetic engineering builds, we must of course pause and ask: is it prudent, and is it ethical?”

our species and which are implicated in disease. Most mutant genes implicated in human disease do not cause disease in every patient that carries that mutation. For reasons scientists are only beginning to unravel, “penetrance”—the extent to which a given phenotype is consistent with a given genotype—is highly variable due to environmental factors, other interacting genes, and cellular factors

Additional complications arise because in some cases, mutations which cause disease in one context confer health advantages in other contexts. The classic example is the gene for sickle cell anemia. Two mutant copies of the sickle cell gene cause disease, but one copy confers resistance to malaria. Taken together, this means that there may be unintended consequences of changing the sequence

of a gene. This is a risk that might be worth taking for an individual patient who is not going to pass that change onto his or her children. It would be cavalier to begin making such changes in germline DNA that will permanently alter the human gene pool.

Third, germline genetic engineering takes us into the murky water of how imprecise definitions of “disease” and “harm” can be. In the field of plastic surgery, for example, therapy and enhancement can be difficult to distinguish. If we begin to allow parents to correct “bad” mutations in their embryos, drawing such distinctions will be even more problematic. Culturally, there is already disagreement—particularly among affected individuals—about whether being deaf or dwarf is truly a disability. What is a liability in some communities is an asset in others. Whatever we might think about physical enhancement, adults who want to “improve” themselves through plastic surgery or other means are making an individual decision about their own body. Children whose genomes are edited as embryos have no such choice. As others have extensively argued, allow-

ing parents to choose the characteristics of their children implicitly commodifies children and subverts their dignity.

Furthermore, germline genetic engineering, even if made widely available in the developed world, will exacerbate preexisting global socioeconomic inequities. The developed world would not only be healthier in terms of nutrition and decreased risk from pathogen-based disease, but would also be on the path to becoming genetically superior,

against the existing patients who deserve respect, dignity, and support.”²⁰

Fifth, although the most commonly stated reason for pursuing germline genetic engineering is of course the possibility of easing human suffering and even curing disease, in most cases, germline genetic engineering is not medically necessary. For many diseases, although this has only been shown in principle for a few, therapy could in theory be accomplished by reprogramming cells from affected

“...in most cases, germline genetic engineering is not medically necessary.”

in an exponential fashion. Similarly, the dignity of disabled individuals will be put at additional risk. As some Japanese researchers recently argued, “If child-birth with a genetic disease no longer occurs in a country due to the extensive practice of the preventive medicine [that is, germline genetic modification], it might impact the rights of the disabled with the genetic disease, intentionally or unintentionally assuming a posture

tissues into iPS cells, making the necessary correction in the DNA of those cells, and directing the cells to develop back into the tissue-type of choice. For diseases not amenable to this approach, as MIT biologist Eric Lander argued, “Genome editing would require making IVF embryos, using preimplantation genetic diagnosis (PGD) to identify those that would have the disease, repairing the gene, and implanting the embryo. Yet it



would be easier and safer simply to use PGD to identify and implant the embryos that aren't at risk."²¹

This brings us to a more fundamental moral concern about germline gene editing. Whether the actual correction is made in egg or sperm cells or in the early embryo itself, germline genetic engineering—like human cloning—requires the special creation of IVF embryos and then necessitates the destruction of those in which the editing was ineffective or those which are simply not needed. Treating human embryos as products to be made and discarded is an assault on human dignity.

In spite of these serious ethical concerns, Congress has never banned germline genetic engineering, although the federal prohibition on the use of federal funding for research in which human embryos are harmed or destroyed (the Dickey-Wicker amendment) remains in place.²² National Institutes of Health (NIH) director Francis Collins said, "NIH will not fund any use of gene-editing technologies in human embryos. The concept of altering the human germline in embryos for clinical purposes has been debated over many years from many different perspectives, and has been viewed

ally created or modified to include a heritable genetic modification,"²⁶ which effectively prohibits any clinical therapies based on germline genetic engineering from being developed in the U.S., along with other techniques that create heritable changes (like mitochondrial transfer, or "3-parent babies"). This language, like the Dickey-Wicker amendment, must be renewed annually.

Mutual Concerns

Unlike the debate about embryonic stem cell research, which was quickly recast into the familiar lines of the abortion debate, the controversy surrounding germline genetic engineering raises an additional set of questions that trouble many scientists who are not pro-life. This ambivalence amongst the scientific community may result in a surprising degree of "self-policing." Last year, *Technology Review* reported that Nessian Bermingham, CEO of Intellia Therapeutics, "a Boston startup that raised \$15 million last year to develop CRISPR into gene therapy treatments for adults or children. . . says germline engineering 'is not on our commercial radar,' and he suggests that his company could use its patents to prevent anyone from commercializing it."²⁷

we use not only for view or rareness, but likewise for dissections and trials; that thereby we may take light what may be wrought upon the body of man. . . . By art likewise, we make them greater or taller than their kind is; and contrariwise dwarf them, and stay their growth: we make them more fruitful and bearing than their kind is; and contrariwise barren and not generative. Also we make them differ in colour, shape, activity, many ways. We find means to make commixtures and copulations of different kinds; which have produced many new kinds, and them not barren, as the general opinion is. . . . Neither do we this by chance, but we know beforehand, of what matter and commixture what kind of those creatures will arise."²⁸

Over 200 years later, Mendel began to lay the modern scientific foundation for genetic engineering. Since its inception, scientists, ethicists, and concerned citizens have been wrestling with whether genetic engineering constitutes "playing God." Modern debates about embryonic stem cell research, cloning, three-parent babies, genetically modified food, and synthetic biology expose fundamental philosophical differences among us about what it means to be human and the very purpose of scientific discovery. In the West, the stage for this debate was set during the Enlightenment. Are we the masters of our own destiny? Or are we image-bearing creatures in the service of a more glorious King? Does science enable human flourishing or human mastery?

Does science enable human flourishing or human mastery?

almost universally as a line that should not be crossed."²³ Last year, the Obama administration said that "altering the human germline for clinical purposes is a line that should not be crossed at this time,"²⁴ leaving the door open for germline gene-editing for research purposes. The regulation of germline gene-editing for clinical use falls under the jurisdiction of the FDA, even in its investigational stages, but gene-editing research designed to answer basic questions about human development or infertility does not.²⁵ Congress added new restrictions late last year prohibiting the FDA from reviewing applications for new therapies "in which a human embryo is intention-

The Age-Old Quest for Mastery over Nature

As the writer of Ecclesiastes said, there is, indeed, nothing new under the sun. Modern genetic engineering is at its essence merely one of latest frontiers in a centuries-old quest to gain mastery over nature itself. Although the techniques were unavailable at the time, Sir Francis Bacon, who many consider to be the father of the scientific method, envisioned a utopia brought about by scientific discovery in which:

We have also parks and enclosures of all sorts of beasts and birds which

These historical details are not an entertaining side-bar to the CRISPR/Cas9 story. Rather, the philosophical traditions we have inherited bear directly on the trajectory of this and other current bioethical debates. Recognizing these historical influences can help us move beyond polarizing rhetoric and instead marshal arguments that resonate in our current cultural context. The ambivalence many scientists feel about human germline engineering may reflect a fundamental sense that there is more to being human than they are able to articulate. Shared values of human flourishing, equity, and justice may give us tools to

use in the public square to persuade others in our pluralistic society that permanently modifying the human genome is not in the interest of our common good.



- 1 David Cyranoski and Sara Reardon, "Chinese Scientists Genetically Modify Human Embryos," *Nature*, April 22, 2015, <http://www.nature.com/news/chinese-scientists-genetically-modify-human-embryos-1.17378> (accessed March 10, 2016).
- 2 Ewen Callaway, "UK Scientists Gain Licence to Edit Genes in Human Embryos," *Nature* 530, no. 7588 (2016): 18.
- 3 Matthew Harper, "This Protein Could Change Biotech Forever," *Forbes*, March 19, 2013, <http://www.forbes.com/sites/matthewharper/2013/03/19/the-protein-that-could-change-biotech-forever/#4bda2b6c473b>.
- 4 Maywa Montenegro, "CRISPR Is Coming to Agriculture—with Big Implications for Food, Farmers, Consumers, and Nature," *Ensisia.com*, January 28, 2016, <http://ensisia.com/voices/crispr-is-coming-to-agriculture-with-big-implications-for-food-farmers-consumers-and-nature/>.
- 5 Harry Glorikan, "Gene Editing Will Change Everything—Just Not All at One Time," *GenEngNews*, January 2, 2015, <http://www.genengnews.com/insight-and-intelligence/gene-editing-will-change-everything-just-not-all-at-one-time/77900351/>.
- 6 Michael Firko, "Letter Bing Yang," United States Department of Agriculture, May 22, 2015, https://www.aphis.usda.gov/biotechnology/downloads/reg_loi/aphis_resp_isu_ting_rice.pdf (accessed March 21, 2016). Other similar letters are available at: <https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/am-i-regulated/regulated+article+letters+of+inquiry/regulated+article+letters+of+inquiry>.
- 7 Yuyu Niu et al., "Generation of Gene-Modified Cynomolgus Monkey via Cas9/RNA-Mediated Gene Targeting in One-Cell Embryos," *Cell* 156, no. 4 (2014): 836–843.
- 8 Hao Yin et al., "Genome Editing with Cas9 in Adult Mice Corrects a Disease Mutation and Phenotype," *Nature Biotechnology* 32, no. 6 (2014): 551–553.
- 9 Hannah Osborne, "Malaria and Zika: CRISPR Gene Editing Could Wipe Out Blood-sucking Female Mosquitoes," *International Business Times*, February 17, 2016, <http://www.ibtimes.co.uk/malaria-zika-crispr-gene-editing-could-wipe-out-blood-sucking-female-mosquitos-1544426>.
- 10 Paula Rio et al., "Targeted Gene Therapy and Cell Reprogramming in Fanconi Anemia," *EMBO Molecular Medicine* 6, no. 6 (2014): 835–848.
- 11 Pankaj K. Mandal et al., "Efficient Ablation of Genes in Human Hematopoietic Stem and Effector Cells Using CRISPR/Cas9," *Cell Stem Cell* 15, no. 5 (2014): 643–652.
- 12 Department of Health and Human Services, "Recombinant DNA Research: Actions under the Guidelines," *Federal Register* 60, no. 81 (1995), 20726–20737, <https://www.gpo.gov/fdsys/pkg/FR-1995-04-27/html/95-10381.htm> (accessed March 9, 2016).
- 13 American Medical Association, "Opinion 2.11—Gene Therapy," issued Dec, 1988, updated June 1994 and June 1996, <http://www.ama-assn.org/ama/pub/physician-resources/medical-ethics/code-medical-ethics/opinion211.page> (accessed March 9, 2016).
- 14 Puping Liang et al., "CRISPR/Cas9-Mediated Gene Editing in Human Trippronuclear Zygotes," *Protein Cell* 6 no. 5 (2015): 363–372. See also Cyranoski and Reardon, "Chinese Scientists Genetically Modify Human Embryos."
- 15 Antonio Regalado, "Engineering the Perfect Baby," *MIT Technology Review*, March 5, 2015, updated April 23, 2015, <https://www.technologyreview.com/s/535661/engineering-the-perfect-baby/> (accessed March 9, 2016).
- 16 Edward Lanphier et al., "Don't Edit the Human Germ Line," *Nature* 519, no. 7544 (2015), 410–411.
- 17 Regalado, "Engineering the Perfect Baby."
- 18 David Baltimore et al., "A Prudent Path Forward for Genomic Engineering and Germline Gene Modification," *Science* 348 (2015): 36–38.
- 19 Callaway, "UK Scientists Gain Licence to Edit Genes in Human Embryos," 18.
- 20 Motoko Araki and Tetsuya Ishii, "International Regulatory Landscape and Integration of Corrective Genome Editing into In Vitro Fertilization," *Reproductive Biology and Endocrinology* 12, no. 108 (2014), doi:10.1186/1477-7827-12-108.
- 21 Eric S. Lander, "Brave New Genome," *The New England Journal of Medicine* 373, no. 1 (2015): 5–8.
- 22 The Balanced Budget Downpayment Act, Pub. L. No. 104–99, Section 128 (1996).
- 23 Francis S. Collins, "Statement on NIH Funding of Research Using Gene-Editing Technologies in Human Embryos," NIH Director Page, April 29, 2015, <http://www.nih.gov/about-nih/who-we-are/nih-director/statements/statement-nih-funding-research-using-gene-editing-technologies-human-embryos> (accessed March 11, 2016).
- 24 John P. Holdren, "A Note on Gene Editing," White House Blog, published May 26, 2015, <https://www.whitehouse.gov/blog/2015/05/26/note-genome-editing> (accessed March 11, 2016).
- 25 President's Council on Bioethics, "Research Involving In Vitro Human Embryos" in *Reproduction and Responsibility: The Regulation of New Biotechnologies* (2004), <https://bioethicsarchive.georgetown.edu/pcbe/reports/reproductionand-responsibility/chapter5.html> (accessed March 10, 2016).
- 26 Agriculture, Rural Development, Food and Drug Administration, and Related Agencies Appropriations Act of 2016, H.R. 3049, 114th Cong. (2016).
- 27 Regalado, "Engineering the Perfect Baby."
- 28 Francis Bacon, *The New Atlantis*. 1627. Project Gutenberg, <http://www.gutenberg.org/files/2434/2434-h/2434-h.htm> (accessed March 9, 2016).

QUESTIONS?

Would you like to offer comments or responses to articles and commentaries that appear in *Dignitas*? As we strive to publish material that highlights cutting-edge bioethical reflection from a distinctly Christian perspective, we acknowledge that in many areas there are genuine disagreements about bioethical conclusions. To demonstrate that bioethics is a conversation, we invite you to send your thoughtful reflections to us at info@cbhd.org with a reference to the original piece that appeared in *Dignitas*. Our hope is to inspire charitable dialogue among our readers and those who contribute material to this publication.