Review

CRISPR Ethics: Moral Considerations for Applications of a Powerful Tool

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Abstract

With the emergence of CRISPR technology, targeted editing of a wide variety of genomes is no longer an abstract hypothetical, but occurs regularly. As application areas of CRISPR are exceeding beyond research and biomedical therapies, new and existing ethical concerns abound throughout the global community about the appropriate scope of the systems' use. Here we review fundamental ethical issues including the following: 1) the extent to which CRISPR use should be permitted; 2) access to CRISPR applications; 3) whether a regulatory framework(s) for clinical research involving human subjects might accommodate all types of human genome editing, including editing of the germline; and 4) whether international regulations governing inappropriate CRISPR utilization should be crafted and publicized. We conclude that moral decision making should evolve as the science of genomic engineering advances and hold that it would be reasonable for national and supranational legislatures to consider evidence-based regulation of certain CRISPR applications for the betterment of human health and progress.

Introduction

The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-Cas9 (CRISPR-associated protein 9) system ("CRISPR" or "the system") is the most versatile genomic engineering tool created in the history of molecular biology to date. This system's ability to edit diverse genome types with unprecedented ease has caused considerable excitement and alarm throughout the international biomedical community.

CRISPR appears to offer considerable promise in a wide variety of disease contexts. For example, around the world at least 15 clinical trials—focused on multiple myeloma; esophageal, lung, prostate, and bladder cancer; solid tumors; melanoma; leukemia; human papilloma virus; HIV-1; gastrointestinal infection; β-thalassemia; sickle-cell anemia; and other diseases—involving CRISPR applications have been developed [1–3]. Moreover, as of May, 2018, in China at least 86 individuals have had their genes altered as part of clinical trials [4].

While significant public support exists for therapeutic applications [5], ethical (moral) and safety concerns about certain areas of CRISPR applications, such as germline editing, are apparent around the world [6]. Notably, such discussions commenced during the Napa Valley meeting of 2015 when a leading group of CRISPR–Cas9 developers, scientists, and ethicists met to examine the biomedical, legal, and ethical aspects of CRISPR systems [7]. From this meeting, more extensive deliberations were solicited, and the United States (US) National Academies of Sciences, Engineering, and Medicine (NASEM or "The Committee") invited the Chinese Academy of Sciences and the United Kingdom's (UK) Royal Society to participate in the International Summit on Human Gene Editing [8]. The goal of this meeting was to examine when, where, and how the technology might be applied in humans. This discussion continued in February of 2017 when a multidisciplinary committee of the NASEM published a comprehensive report examining numerous aspects of human genome editing [9].

To date, the NASEM report provides perhaps the most influential, extensive analysis examining wide-ranging concerns about human genome editing [10]. Importantly, the Committee favored somatic genome editing, but did not permit genomic modification for any kind of enhancement [9, 11]. Also, though impermissible at present, the Committee concluded cautiously that human heritable genome editing, the modification of the germline with the goal of creating a new person who could potentially transfer the genomic edit to future generations, would be permissible under certain conditions: "In light of the technical and social concerns involved … heritable genome-editing research trials might be permitted, but only following much more research aimed at meeting existing risk/benefit standards for authorizing clinical trials and even then, only for compelling reasons and under strict oversight." [9] Although by law, US federal funding cannot be used to support research involving human embryos [12–14], the NASEM report suggests that when technical and safety risks are better understood then clinical trials involving germline editing might begin [9].

In this review, we aim to summarize fundamental ethical concerns about CRISPR use in general, but the list is not exhaustive. First, we briefly review CRISPR systems and their applications in editing genomes and epigenomes. Second, we describe how complexities of CRISPR science affect those of CRISPR ethics and *vice versa*. Third, we assess several key ethical considerations. Notably, while some of these concerns are specific to CRISPR technology, many, such as research on human embryos, have been debated long before the CRISPR revolution [15]. Moreover, since CRISPR is still a maturing technology, novel applications in the future may raise new ethical quandaries meriting further attention and dissection. Fourth, it is important to point out that, though morality and law often overlap, significant differences exist. Although law may affect ethics and *vice versa*, we focus mostly on *ethics*. Finally, while discussing these issues, we assume no position on any topic; our account is merely *descriptive*. Therefore, we make no attempt to *settle* any of the controversies presented herein.

CRISPR systems and their uses

Different CRISPR systems in genome editing

CRISPR is a natural bacterial defense system against invading viruses and nucleic acids. Over billions of years, multiple CRISPR-type immune systems have evolved. Naturally occurring CRISPR systems are typically classified by their repertoires of CRISPR-associated (*cas*) genes, which are often found adjacent to the CRISPR arrays [16, 17]. Although the characterization is yet to be finalized, two major classes of CRISPR–Cas adaptive immune systems have been identified in prokaryotes [18–20]. This division is based on the organization of effector modules. **Class 1** CRISPR–Cas systems employ multi-protein effector complexes and encompass three types (I, III, and IV). By contrast, **Class 2** systems utilize single protein effectors and encompass three other types (II, V, and VI). Although various natural CRISPR–Cas systems have been repurposed for genome editing, due to its robust gene-editing efficiency and broader genome-targeting scope owing to its simple NGG PAM sequence requirement, the Cas9 from *Streptococcus pyogenes* (spCas9) is currently the most commonly used CRISPR–Cas9 protein. It is worth noting that multiple efforts are underway to discover novel Cas9 variants or re-engineer the existing Cas9 proteins, which will have less dependence on the stringent PAM-sequence requirement [21, 22].

CRISPR goes beyond genome editing

The DNA-editing capacity of CRISPR–Cas9 is due to the ability of the WT Cas9 protein to cause double-stranded breaks at the target site that is determined by the custom-designed short guiding RNA [23]. The repair of DNA breaks frequently results in indels, due to the non-homologous end joining (NHEJ) repair mechanism. However, when a complementary template is available, homology-directed repair (HDR) machinery can use it and thereby achieve more precise gene editing. Notably, a single-point mutation in either of the two catalytic domains of Cas9 results in a nickase Cas9 (nCas9), whereas mutations in both domains (D10A and H840A for spCas9) diminish Cas9's catalytic activity, resulting in dead Cas9 (dCas9) [24]. Interestingly, the application areas of modified Cas9 proteins are exceeding that of WT Cas9 [25]. Such uses are largely possible because the nCas9 or dCas9 can still be guided to the target sequence [26]. Researchers employed these Cas9 variants for unique purposes. For example, tandem targeting of nCas9 has been utilized to improve targeting specificity [27, 28]. More recently, this enzyme has been used as the base platform for second generation genome-editing tools called "base editors" [29, 30] Base-editing technology employs cytidine or adenine deaminase enzymes to achieve the programmable conversion of one base into another (C to T or A to G). Most importantly, the targeted base transition happens without DNA double-stranded breaks [29, 31]. We recently utilized this technology to edit the universal genetic code and introduced a "stop" codon in the genes [32].

In addition to nCas9, researchers utilized the guidable capacity of dCas9 as a platform to recruit various effector proteins to a specific locus in living cells. Generally, these activities can be classified as

epigenetic editing (to alter locus-specific epigenetic information), gene regulation (to turn the activities of single or multiple genes on or off), chromatin imaging (to label and monitor chromatin dynamics in living cells), and manipulation of chromatin topology (to alter 3D chromatin structure in the nuclear space) [33].

CRISPR research is progressing at a rapid pace. Recently, scientists have also uncovered new CRISPR–Cas systems (Cas13) that can target RNA instead of DNA [34, 35]. By enabling targeted RNA recognition and editing, these newer RNA-targeting CRISPR tools have their unique applications ranging from biomedical and biotechnological to the detection of nucleic acids [36, 37]. Although many ethical concerns are related to the catalytic activities of WT Cas9—partly because it permanently alters the genetic information—some of these activities of catalytically inactive dCas9, nickase-Cas9-based platforms, such as base editors and recently discovered RNA-targeting Cas proteins, may raise comparable moral issues depending on the duration of the exerted effect and the purpose of the experiments. Detailed discussion of such issues, however, is beyond the scope of this review (Table 1).

CRISPR ethics and science: Uncomfortable bedfellows

Moral decisions, especially in biomedicine, are empirically informed and involve assessing potential risk-benefit ratios—attempting to maximize the latter while minimizing the former. To navigate ethical decision making, it is critical to consider the range of possible outcomes, the probabilities of each instantiating, and the possible justifications driving the results of any one. The ethical concerns about CRISPR genome engineering technology are largely due to at least three important reasons.

First, there are concerns about the power and technical limitations of CRISPR technology. These include the possibilities of limited on-target editing efficiency [38, 39], incomplete editing (mosaicism) [40, 41], and inaccurate on- or off-target editing [42, 43]. These limitations have been reported in CRISPR experiments involving animals and human cell lines. However, the technology is evolving at an unprecedented pace. As more efficient and sensitive CRISPR tools are developed, many of these concerns may become obsolete. Yet for the sake of this review, we mention these limitations as one of the principal worries about widespread CRISPR utilization. Second, it is unclear whether modified organisms will be affected indefinitely and whether the edited genes will be transferred to future generations, potentially affecting them in unexpected ways. Combined with technical limitations and the complexities of biological systems, making precise predictions about the future of an edited organism and gauging potential risks and benefits might be difficult, if not impossible. Thus, uncertainty resulting from these factors hinders accurate risk-benefit analysis, complicating moral decision making.

Finally, the skeptical view is that even if the genome is edited as expected and the desired functional output is achieved at the given time, the complex relationship between genetic information and biological phenotypes is not fully understood. Therefore, the biological consequence of editing a gene in germline and/or somatic cells may be unclear and unpredictable depending on the context. Many biological traits are determined by the complex regulatory actions of numerous genes. Hence, is it difficult, if not impossible, to “design” a biological phenotype at the whole-organism level. Across biological outcomes, whether in normal or in disease development, it is uncommon that a single gene is *the only factor* shaping a complex biological trait. Other genetic regulatory factors such as additional genes or distal regulatory elements (e.g., enhancer or repressor elements), as well as environmental and epigenetic factors, contribute to the emergence of a biological phenotype. To argue that modifying a gene changes a desired phenotype (under certain conditions) implies at least a reasonable understanding of other independent variables contributing to the phenotype's instantiation. But this understanding is still far from complete in many normal and disease processes [44, 45]. Given the uncertainty regarding how gene expression and modification influence complex biological outcomes, it is difficult to appraise potential risk and benefit. This ambiguity creates a challenge on its own and is one of the sources obscuring efficient ethical deliberation and decision making.

Nevertheless, regulations governing cellular- and gene-therapy research may facilitate the safe development and oversight of some clinical trials involving CRISPR-based-editing applications. In the United States, for instance, cellular- and gene-therapy products, including many CRISPR applications, at this time are defined generally by the Food and Drug Administration as *biological products* and are regulated by the Food and Drug Administration's Center for Biologics Evaluation and Research/Office of Cellular, Tissue, and Gene Therapies [46–48]. Although the risks and benefits of many such therapies increasingly are better understood [49], questions regarding safety and efficacy remain. Thus, future advancements likely will continue to improve the benefits of this revolutionary technology, while minimizing the potential risks. Regardless of the uncertainty posed by novel CRISPR technologies and applications, in several locations around the world significant regulatory frameworks exist by which risks may be monitored and contained. However, wherever such infrastructure and oversight are lacking, safety and privacy risks might increase.

Table 1. Risk-benefit considerations in CRISPR technology

	Benefit(s)	Risk(s)/Harm(s)
Basic and pre-clinical research	• New model organisms and cell lines • Increased gene-editing efficiency • High-throughput screens • Novel drug targets • Access to totipotent cells • Identification of novel signaling, regulatory, and developmental pathways • Development of novel gene-editing approaches (base editing and RNA targeting) • Knowledge advancement	• Experimentation involving human embryos is controversial and illegal in some countries • Potential for privacy and confidentiality breaches
Translational and clinical medicine	• Immunotherapy • Organoids • Novel drug targets • Artificial intelligence • Modification of pathological genes • Novel therapeutics and fertility applications • Procreative liberty • Ability to "fix" single base changes • Knowledge advancement • Potential for equitable access	• Serious injury, disability, and/or death to research participant(s) and/or offspring • Blurry distinction between therapeutic and enhancement applications, leading to potential subtle or obvious exacerbation of inequalities • Misapplications • Eugenics • Potential for inequitable access and exacerbation of inequalities
Non-therapeutic applications	• Enhancement to augment select faulty or normal human characteristics • Fortification of crops and livestock • Successful control of pests, invasive species, and reservoirs (gene drives) • Disease/infection control (e.g., malaria, dengue fever, Lyme and Chagas disease, schistosomiasis) • Ecosystem alteration to protect endangered species (gene drives) • Safety • Crop cultivation • Knowledge advancement	• Eugenics • Exacerbation of racism and inequality • Theoretical risk for damage to ecosystems • Theoretical risk of misuse
Access to CRISPR technology	• Inexpensive (technology itself) • Widely available • Profit, economic growth • Innovation	• Price gouging • Prohibitively expensive applications
Regulations for clinical research involving human subjects	• Established framework in some countries to manage research risks • Legal mechanisms for redress already exist, depending on location	• Lack of appropriate supervisory infrastructure, oversight, and/or regulatory framework in many nations • Unclear how to supervise the research even in some countries with regulatory oversight • Over-regulation might hinder progress
National and international regulations, law, and policy	• Prevention against misuses of technology • Safeguard against risky, potentially harmful conditions	• Potential to encroach on individual, scientific, and societal autonomy • Limit discovery and progress • Difficult enforcement • Lack of uniformity may create inconsistencies in applications of laws/regulations

Ethical concerns

To what extent should CRISPR experimentation be permitted in basic and pre-clinical biomedical research?

Although it is less than a decade old, CRISPR–Cas9 has demonstrated unprecedented potential to revolutionize innovation in basic science. From viruses and bacteria [50, 51], to simple model organisms, such as *Drosophila melanogaster* (fruit fly) [52], *Anopheles gambiae* (mosquito) [53], *Saccharomyces cerevisiae* (budding yeast) [54], *Hydra magnipapillata* (hydra) [55], *Caenorhabditis elegans* (round worm) [56], *Danio rerio* (zebra fish) [57], and *Arabidopsis thaliana* (rockcress) [58], to larger animals such as pigs [59], cattle [60], and monkeys [61], and even human

zygotes [62, 63], CRISPR experimentation has led to novel, important findings. Such benefits include at least the following: increased overall efficiency in gene editing compared with previous genomic engineering techniques like transcription activator-like effector nucleases (TALENs) and zinc finger nucleases (ZNFs) [64]; significant insights into the evolutionary transformation of fish fins into tetrapod limbs [65]; investigation into new organisms [66]; genetic and epigenetic screens [67, 68]; the creation of novel cell lines [69]; high-throughput screens and libraries [70]; the elucidation of novel genomic and epigenomic regulatory pathways [71, 72]; insights into the development of butterfly coloring and patterning [73]; the functional characterization of key genes and molecular signaling pathways [74, 75]; and drug-targeting screens [76, 77]. Data from such experimentation provide essential clues and understanding that promote biomedical discovery, advancement, and the basis for potential medical benefits.

One of the major controversies about CRISPR technology emerges from its possible application in human embryos. This controversy is not about CRISPR itself, but instead is largely due to the lack of clarity about the status of the human embryo. Although some in the scientific community maintain that it is ethically impermissible to experiment on human embryos after 14 days [78, 79], it is impossible for any one party—whether it is a government, laboratory, funding agency, panel of experts, court, religious organization, or other group—to decide the status of a human embryo [80, 81] and whether and precisely when it has "personhood" [82]: Is the entity merely a ball of cells whose status is like that of human skin, which sheds regularly without further ado? Or does the entity hold complete personhood status—with irreducible, inalienable moral rights and to whom we owe important directed duties? Or is the embryo's status characterized optimally as something in between? And if so, which moral rights might this entity hold, and which duties might we owe to it? Despite this perplexing complexity, decisions one way or the other must be executed, because proceeding with research or failing to do so has important consequences: Banning or significantly limiting research on human embryos stymies progress at least by making unavailable or curtailing an option to investigate the therapeutic potentials of stem cells and the biology of totipotent cells, which currently are not known to be present in any other viable human tissue sources. Totipotent cells can divide indefinitely and have the capacity to develop into all tissue types. Depending on how the status of the embryo is appraised, however, the ban also could save it from potentially unjust, lethal research-related harms. Even if the research is justified because of its potential benefit to the embryo itself and/or to others, the embryo as such cannot give informed consent at the time of the research, since the entity is not sufficiently developed. But from the research, it could experience potentially life-altering consequences—good or bad—that may extend throughout the lifespan and future generations.

By contrast, promoting such investigation may facilitate the development of novel *in vitro* fertilization techniques and advances for conditions such as spinal cord injury [83], Parkinson's disease [84], burns [85], cardiomyopathy [86], and other ailments that might be ameliorated by approaches involving regeneration. Taken together, countries must continue to decide as the science progresses whether and how to legalize experimentation on human embryos. Current positions across the globe vary widely—from outright banning of the research to illegalizing its federal funding only (while still allowing private funding for research and the research itself) to authorizing federal monies for experimentation [4, 6, 87, 88].

To what extent should CRISPR use be permitted in translational and clinical medicine?

CRISPR is significantly benefitting, and is likely to improve, immunotherapy [89], organoid engineering and development [90], *in vivo* drug target identification [91], machine learning and artificial intelligence [92], and disease-gene modification in viable human embryos [62]. The system offers nearly boundless potential to promote progress in combating HIV [93], hemophilia [94], cancer [95], Duchenne muscular dystrophy [96], amyotrophic lateral sclerosis [97], sickle-cell anemia [98], cystic fibrosis [99], infertility [100], and any number of novel diseases. The potential both for gaining knowledge and for developing treatments in humans seems nearly endless.

However, such knowledge and treatment acquisition are not without potential risk. With experimentation involving somatic cells, risk assessment seems at least comparable to that which arises in regularly practiced biomedical testing. The chief objective of phase-2 oncology trials, for example, is to evaluate the efficacy of a new drug or device [101]. Study participants may assume significant harms, including possible irreversible side effects and death [102, 103]. In many countries, respect for autonomy permits assuming such risk with the requirement that informed consent occurs before enrollment in the research, regardless of whether individuals are enrolling themselves or their dependents. If this risk is considered morally (and legally) permissible, then it would seem unjustified and unreasonable to not allow risk posed by investigations involving CRISPR-based genome engineering. At the time of this writing, there is no empirical support suggesting that CRISPR experimentation would necessarily pose greater risk: the overall risk profile of CRISPR experimentation in human subjects remains unknown.

It could be argued, however, that heritable germline editing might present *additional* risk, because it involves not only the research participant but also

potentially his or her descendants. Of course, whether germline engineering technologies introduce risk beyond that which might be present in more common testing scenarios is an empirical matter. For instance, it is well established that routinely used chemotherapies have mutagenic properties: alkylating agents, including cisplatin and cyclophosphamides, cause DNA adducts and crosslinks; antimetabolites, such as hydroxyurea, gemcitabine, and 5-fluorouracil, are nucleoside analogs and inhibit thymidine synthase; topoisomerases, such as etoposide, cause topoisomerase II inhibition, leading to double-stranded breaks in DNA; and anthracyclines, like doxorubicin, cause DNA intercalation [104]. Therefore, significant exposure to any of these agents increases the probability of both incurring genetic mutations and passing on these unintended genomic alterations to future generations. Whether the risk level presented by such exposure is greater than, equal to, or less than that presented by CRISPR experimentation must be quantitatively determined by empirical evidence. It is also an empirical matter whether CRISPR introduces risk that is statistically significant beyond that which is incurred in the daily experience of a healthy individual with little-to-no exposure to mutagenic agents. Thus, to determine with confidence whether it is exceptionally risky to involve humans in CRISPR translational and clinical research, possible research-related risks must be compared with those in other potentially dangerous experimental and every-day contexts. This is difficult, however, given that CRISPR technology is new and that robust, reliable data about CRISPR risk in human subjects are unavailable. Nevertheless, decision making about assuming risk in studies and therapeutics should be considered according to legal infrastructure, national and possibly international regulatory agencies, and ultimately navigated by research participants and/or their legally authorized representative(s).

Important questions also arise about whether experiments involving heritable germline editing yield reliable, interpretable data. One objection is that such experiments are unlikely to be controlled and/or predicted [105] because it could be impossible to analyze or understand the results from such experimentation until considerable time (decades or even generations) passes [106]. As previously noted, the central concern here is the uncertainty in the causal connection between gene expression/modification and the potential involvement of other factors shaping biological outcomes in the future.

Another risk, shared globally, is posed by the greater society. It is possible, for instance, that allowing CRISPR germline editing, even if only for medical purposes, might in some respect(s) lead to the return of eugenics, whose proponents believed that the human population can be improved by controlled breeding to increase the occurrence of "desirable", heritable characteristics [107]. Unfortunately, historically, this selective weeding of people with "bad" genes and breeding of those with "good" ones resulted in many atrocities, including the forced sterilization of individuals and the propagation of racially discriminatory policies—both of which were backed by state authorities and even educated elites in different societies. In the notorious case *Buck v. Bell* [108], for example, the United States Supreme Court ("the Court") upheld a Virginia statute permitting the compulsory sterilization of individuals, such as Carrie Buck, who were considered "mentally unfit." Buck was an economically disadvantaged woman who was labeled as "feebleminded" like her other family members of past generations. She was committed to the Virginia Colony for Epileptics and Feeble-Minded and was forcibly sterilized [109]. Unfortunately, however, the evidence of the case strongly indicates that Buck, like the others in her family, was normal and that the Court erred gravely [110]. Its decision, authored by eugenics proponent Associate Justice Oliver Wendell Holmes, led to the sterilization of 50,000 Americans, set a precedent for the Nazi racial hygiene program, and is yet to be overturned [111]. Hence, history reveals that egregious, systematic mistakes are always possible.

To what extent should CRISPR use be permitted for non-therapeutic purposes?

Important ethical questions also arise in non-therapeutic contexts including enhancement of crops, livestock, gene drives, and human features [112].

Certain areas of CRISPR applications, such as the enhancement of crops and livestock, are likely to significantly impact society and humanity at large. In 2016, the United Nations Food and Agriculture Organization estimated that 795 million people in the world were undernourished [113]. And according to the World Health Organization, 2 billion people are unable to obtain key nutrients like iron and vitamin A [114]. Abundant evidence demonstrates that CRISPR–Cas systems could be used to improve nutrient content in foods [106, 115–123]. In principle, CRISPR has the potential to fortify foods efficiently for individuals who are suffering from a lack of basic nutrients. Why not decrease malnutrition by maximizing access to foods of higher quality? Promoting benefit in this way carries moral weight at least comparable to any other ethical concern raised herein, especially given the very large number of those who are nutrient-deprived. Nevertheless, with this benefit arise worries about "accessibility" to these product(s); this issue is discussed further below.

"Gene drive technology" is another CRISPR application with unprecedented potential to directly benefit and save millions of lives [124]. In using gene drives, researchers employ CRISPR to speed up genetic recombination such that a "gene of interest" is rapidly distributed to the entire population much faster than in

a typical Mendelian inheritance rate. Therefore, this application has the potential to edit the genome of an entire population or even an entire species. Using CRISPR-mediated gene drives, investigators have demonstrated that a gene allele providing a parasite-resistance phenotype in mosquitos could quickly spread through the population in a non-Mendelian fashion [111, 125]. This highly cost-effective technology has many potential benefits and applications for public health, species conservation, agriculture, and basic research [126]: Gene drives may provide a fundamental tool to fight against deadly diseases such as malaria [127–129], dengue fever [130], Chagas and Lyme disease [131], and schistosomiasis [132]. This application also may control and/or alter a wide variety of animals (e.g., rodents and bats), invasive plant pests, and reservoirs [133, 134]. Thus, the technology has unprecedented power that may save millions of lives each year. However, it is also important to consider the expected and unexpected risks. Once applied, gene drives will eventually affect every individual of the entire species. Knowing this, researchers are developing and incorporating key safety "off-switch" features such as novel ways to (i) control, (ii) inhibit, and (iii) reverse/eliminate gene drive systems from the population in case of an unexpected or emergency event [134–137].

Furthermore, were it possible, would it be it morally permissible to employ CRISPR techniques to enhance human features such as height, muscle mass, vision, or cognitive factors like learning aptitude and memory? Answering this question is problematic largely because of the difficulty with deciding about what "counts" as pathology vs. what is merely a minor or even moderate deviation from the "norm" in a given context. Moreover, accurately characterizing norms in the first place is often very difficult. Hence, "medical necessity" often becomes ambiguous, and the boundary between "therapy" and "enhancement" can be murky. For example, a gene-editing approach may allow for a reduction in bad cholesterol, thus leading to a healthier life style. Whether this hypothetical scenario, which may benefit both the individual and society in the long run, should be classified as enhancement or a medical need is unclear.

Aside from concerns about well characterizing medical necessity, positive moral liberties are granted and backed by legal rights in many countries, especially in the West. Should medical enhancement by CRISPR technologies be considered a form of free speech and/or expression? [138] If so, how, if at all, might these rights be limited, and why? Who has and/or should have the authority to decide?

Who should have access to CRISPR technology and/or its products?

Benefits from CRISPR innovation raise concerns and controversies about fairness and distributive justice across all layers of society. These matters are not specific to CRISPR technology, but may apply to all other technologies arising from academic research. Like many novel biomedical advancements, new CRISPR applications are expected to be profitable for patent holders. At least the initial prices of CRISPR-based products, such as gene therapy, are likely to be costly [139]. To this end, an ethical question is whether the high price-tag will make the CRISPR product available to only the world's elites. Since much of the funding for CRISPR characterization and development was provided by grants from government funds and thus taxpayers' money [140–151], it is morally problematic to deny potentially lifesaving benefits of the technology to the very individuals who funded much of its development in the first place. Moreover, even if it were affordable for some, there may be economic harms associated with high-price purchases. For instance, those needing CRISPR-based applications to maintain a reasonable quality of life, or even life itself, might be forced to make painful economic choices about whether to spend funds on therapuetics, food, or other essential living necessities. While this problem is not unique to genomic engineering advancements, allowing price gouging to continue unaddressed is unhelpful and potentially allows physical, psychological, and economic harms to continue. Encouraging the establishment of anti-price-gouging laws, where possible, could ameliorate some of these concerns [152].

As discussed in the previous section, CRISPR may be used to fortify foods. Those residing in some of the most impoverished areas of the world are positioned to benefit the greatest from these products. How might such individuals gain access to CRISPR-modified foods, especially in places with armed combat and rogue governments? How, if at all, could companies benefit such that reaching out to these populations might be desirable and/or lucrative?

Limiting human genome editing? Somatic *versus* germline editing

As noted above, obvious applications of CRISPR technology are cell and gene therapies. To date, gene therapy mostly involves the use of genome-engineering technologies to edit somatic cells to treat genetic diseases. Clinical trials involving CRISPR-based gene therapy are already under way. Although clinical gene and cell therapies have had major road blocks in the past, due to unanticipated injuries and death [153, 154], significant safety improvements have been implemented over the last decade [155]. With the advances of CRISPR technology and safer delivery approaches, therapeutic applications of gene therapy are on the rise [156]. In the United States and elsewhere, federal regulations

provide the needed legal and ethical frameworks, through the institutional review board system, to potentially minimize and manage potential risks [156–160].

At present, there is considerable excitement about such experimentation in the United States. In January of 2018, for example, the US National Institutes of Health launched the Somatic Cell Genome Editing program, seeking "to improve the delivery mechanisms for targeting gene editing tools in patients, develop new and improved genome editors, develop assays for testing the safety and efficacy of the genome editing tools in animal and human cells, and assemble a genome editing toolkit containing the resulting knowledge, methods, and tools to be shared with the scientific community" [161].

Heritable genome editing, by contrast, is perhaps the CRISPR systems' greatest discussed controversy. Recently, professional scientific and medical societies, industry organizations, and CRISPR pioneers together have released greater than 60 statements and reports about whether such editing in humans is morally permissible [6]. Most statements hold that heritable germline experimentation should be prohibited currently, although reports from the Netherlands [162], the United Kingdom [163], Spain [164], and the United States [9] suggest that editing could be permissible if certain requirements were satisfied. The NASEM Committee's report on germline editing, for example, specified that the following provisions must be met for human heritable germline research to commence: "the absence of reasonable alternatives; restriction to preventing a serious disease or condition; restriction to editing genes that have been convincingly demonstrated to cause or to strongly predispose to that disease or condition; restriction to converting such genes to versions that are prevalent in the population and are known to be associated with ordinary health with little or no evidence of adverse effects; the availability of credible preclinical and/or clinical data on risks and potential health benefits of the procedures; ongoing, rigorous oversight during clinical trials of the effects of the procedure on the health and safety of the research participants; comprehensive plans for long-term, multigenerational follow up that still respect personal autonomy; maximum transparency consistent with patient privacy; continued reassessment of both health and societal benefits and risks, with broad ongoing participation and input by the public; and reliable oversight mechanisms to prevent extension to uses other than preventing a serious disease or condition" [9]. Although fears about misuse in this context abound, it is important to point out that there are reasonable arguments supporting heritable germline editing in research, such as the protection of defective embryos [165], the elimination of certain diseases that might be obliterated optimally early in embryonic development, and the exercise of free speech and/or expression [138].

Should international regulations governing CRISPR use be crafted and promulgated?

Although ethics statements are important, by themselves, they provide little force. Typically, if ethics guidelines are infringed, the consequences suffered by the violator(s) are fairly minimal compared to those arising when in violation of certain laws. Violations of ethics statements may lead to loss of funding, the retraction of a publication(s), job loss, and mistrust among the biomedical community. By contrast, punishments by law may lead to heavy fines and potentially incarceration. Given the significant potential promise, the dark history of eugenics, the potentially serious transgenerational risks, and the theoretical potential for misuse, it is reasonable for the global community to consider instantiating national and supranational regulations, if not revising older agreements such as the Geneva [166] and the United Nations Conventions on Biological and Toxin Weapons, [167] to reflect changes in genomic engineering technologies. While doing so likely will not eliminate all risks, it is arguably one of the few options available to reasonably control and/or minimize them.

Conclusions and future directions

CRISPR technology continues to mature, and existing systems are being engineered to contain innovative capabilities; excitingly new CRISPR systems with novel functions are still being discovered. The potential benefits of such revolutionary tools are endless. However, like any powerful tool, there are also associated risks raising moral concerns. To make truly informed decisions about areas of ethical controversy, well-controlled, reproducible experimentation and clinical trials are warranted. Currently, this is difficult because many international laws discourage or ban such research and/or inhibit its funding for certain types of investigation. Thus, widespread data about benefits and risks are unavailable. It is critical, however, for countries to examine their reasoning behind these prohibitions to ensure that they are not simply arising out of fear and without reasonable justification.

Going forward, many support establishing an organization that will decide how best to address the aforementioned ethical complexities. Recently, a group of European scientists founded the Association for Responsible Research and Innovation in Genome Editing (ARRIGE) to examine and provide guidance about the ethical use of genome editing [168, 169]. Furthermore, Jasanoff and Hurlbut [170] recently

advocated for the development of an international, interdisciplinary "global observatory for gene editing." Briefly, they argued that deliberations about moral issues in gene editing should not be dominated by the scientific community, but instead should include a "network of scholars and organizations similar to those established for human rights and climate change. The network would be dedicated to gathering information from dispersed sources, bringing to the fore perspectives that are often overlooked, and promoting exchange across disciplinary and cultural divides" [170].

As the technology evolves, so will discussions about ethical and legal frameworks circumscribing its uses. The above-mentioned platforms present interesting ideas for furthering debates and potential resolutions. The research and ethical guidelines from national and international organizations, where diverse disciplines of societies contribute, will be critical for federal funding agencies and institutional review boards to enforce and regulate, to minimize the potentials risks and maximize the potential benefits of CRISPR technology. However, it is likely that the enforcement of research laws and ethical guidelines ultimately will be assumed by governments and their legal systems, principal investigators, and institutional review boards.

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Abbreviations used:
CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; Cas9, CRISPR-associated protein 9; NASEM, US National Academies of Sciences, Engineering, and Medicine.

References

[1] D. Cyranoski, CRISPR gene-editing tested in a person for the first time, Nature 539 (2016) 479.
[2] CRISPR to debut in clinical trials, D. Kwon, Israel Molecular Medicine, [cited 2018 May 15] Available from: http://www.molecular-medicine-israel.co.il/crispr-debut-clinical-trials/ 2017.
[3] ClinicalTrials.gov, U.S. National Library of medicine, [cited 2018 May 20]. Available from: https://clinicaltrials.gov/ct2/results?cond=&term=CRISPR&cntry=&state=&city=&dist= 2018.
[4] L. The, Editing the human genome: balancing safety and regulation, Lancet 391 (2018) 402.
[5] D.A. Scheufele, M.A. Xenos, E.L. Howell, K.M. Rose, D. Brossard, B.W. Hardy, U.S. attitudes on human genome editing, Science 357 (2017) 553–554.
[6] C. Brokowski, Do CRISPR germline ethics statements cut it? CRISPR J. 1 (2) (2018) 115–125.
[7] D. Baltimore, P. Berg, M. Botchan, D. Carroll, R.A. Charo, G. Church, et al., Biotechnology. A prudent path forward for genomic engineering and germline gene modification, Science 348 (2015) 36–38.
[8] International Summit on Human Gene Editing: A Global Discussion [Internet]. National Academy of Sciences Engineering and Medicine, U.S., 2015, [cited 2018 Feb 16]. Available from: http://nationalacademies.org/cs/groups/pgasite/documents/webpage/pga_170455.pdf.
[9] National Academies of Sciences Engineering and Medicine (U.S.), Human Genome Editing: Science, Ethics, and Governance, The National Academies Press, Washington, DC, 2017.
[10] Many countries and international decision-making bodies, including the Nuffield Council on Bioethics, the European Society of Human Genetics, and the European Society of Reproduction have issued reports and statements about ethical issues in genomic engineering, focusing specifically on the moral permissibility of germline editing. However, to date, the U.S. NASEM report (2017) is the most robust.
[11] With stringent oversight, heritable germline editing clinical trials could one day be permitted for serious conditions; non-heritable clinical trials should be limited to treating or preventing disease or disability at this time, National Academies of Sciences Engineering and Medicine (News). [2018 May 20]. Available from: http://www8.nationalacademies.org/onpinews/newsitem.aspx?RecordID=24623 2017.
[12] Code of Federal Regulations: Research Involving Pregnant Women or Fetuses, 45 C.F.R. Sect 462017.
[13] United States Code: Institutional Review Boards; Ethics Guidance Program, 42 U.S. Code Sect 2892017.
[14] Consolidated Appropriations Act of 2016 [statute on the internet] c2017, [cited 2017 Nov]. Available from: https://www.congress.gov/114/bills/hr2029/BILLS-114hr2029enr.pdf.
[15] J.A. Robertson, Human embryonic stem cell research: ethical and legal issues, Nat. Rev. Genet. 2 (2001) 74–78.
[16] D.H. Haft, J. Selengut, E.F. Mongodin, K.E. Nelson, A guild of 45 CRISPR-associated (Cas) protein families and multiple CRISPR/Cas subtypes exist in prokaryotic genomes, PLoS Comput. Biol. 1 (2005), e60.
[17] K.S. Makarova, D.H. Haft, R. Barrangou, S.J. Brouns, E. Charpentier, P. Horvath, et al., Evolution and classification of the CRISPR–Cas systems, Nat. Rev. Microbiol. 9 (2011) 467–477.

[18] K.S. Makarova, Y.I. Wolf, O.S. Alkhnbashi, F. Costa, S.A. Shah, S.J. Saunders, et al., An updated evolutionary classification of CRISPR–Cas systems, Nat. Rev. Microbiol. 13 (11) (2015) 722–736.
[19] E.V. Koonin, K.S. Makarova, F. Zhang, Diversity, classification and evolution of CRISPR–Cas systems, Curr. Opin. Microbiol. 37 (2017) 67–78.
[20] S. Shmakov, A. Smargon, D. Scott, D. Cox, N. Pyzocha, W. Yan, et al., Diversity and evolution of class 2 CRISPR–Cas systems, Nat. Rev. Microbiol. 15 (3) (2017) 169–182.
[21] B.P. Kleinstiver, M.S. Prew, S.Q. Tsai, V.V. Topkar, N.T. Nguyen, Z. Zheng, et al., Engineered CRISPR–Cas9 nucleases with altered PAM specificities, Nature 523 (2015) 481–485.
[22] J.H. Hu, S.M. Miller, M.H. Geurts, W. Tang, L. Chen, N. Sun, et al., Evolved Cas9 variants with broad PAM compatibility and high DNA specificity, Nature 556 (2018) 57–63.
[23] H. Nishimasu, F.A. Ran, P.D. Hsu, S. Konermann, S.I. Shehata, N. Dohmae, et al., Crystal structure of Cas9 in complex with guide RNA and target DNA, Cell 156 (2014) 935–949.
[24] L.S. Qi, M.H. Larson, L.A. Gilbert, J.A. Doudna, J.S. Weissman, A.P. Arkin, et al., Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression, Cell 152 (2013) 1173–1183.
[25] M. Adli, The CRISPR tool kit for genome editing and beyond, Nat. Commun. 2018 (9) (1911).
[26] M. Jinek, K. Chylinski, I. Fonfara, M. Hauer, J.A. Doudna, E. Charpentier, A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity, Science 337 (2012) 816–821.
[27] P. Mali, J. Aach, P.B. Stranges, K.M. Esvelt, M. Moosburner, S. Kosuri, et al., CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering, Nat. Biotechnol. 31 (2013) 833–838.
[28] F.A. Ran, P.D. Hsu, J. Wright, V. Agarwala, D.A. Scott, F. Zhang, Genome engineering using the CRISPR–Cas9 system, Nat. Protoc. 8 (2013) 2281–2308.
[29] N.M. Gaudelli, A.C. Komor, H.A. Rees, M.S. Packer, A.H. Badran, D.I. Bryson, et al., Programmable base editing of A*T to G*C in genomic DNA without DNA cleavage, Nature 551 (2017) 464–471.
[30] A.C. Komor, Y.B. Kim, M.S. Packer, J.A. Zuris, D.R. Liu, Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage, Nature 533 (2016) 420–424.
[31] G.T. Hess, J. Tycko, D. Yao, M.C. Bassik, Methods and applications of CRISPR-mediated base editing in eukaryotic genomes, Mol. Cell 68 (2017) 26–43.
[32] C. Kuscu, M. Parlak, T. Tufan, J. Yang, K. Szlachta, X. Wei, et al., CRISPR-STOP: gene silencing through base-editing-induced nonsense mutations, Nat. Methods 14 (2017) 710–712.
[33] A.C. Komor, A.H. Badran, D.R. Liu, CRISPR-based technologies for the manipulation of eukaryotic genomes, Cell 169 (2017) 559.
[34] O.O. Abudayyeh, J.S. Gootenberg, P. Essletzbichler, S. Han, J. Joung, J.J. Belanto, et al., RNA targeting with CRISPR–Cas13, Nature 550 (2017) 280–284.
[35] S.C. Strutt, R.M. Torrez, E. Kaya, O.A. Negrete, J.A. Doudna, RNA-dependent RNA targeting by CRISPR–Cas9, elife 7 (2018).
[36] CRISPR–Cas12a target binding unleashes indiscriminate single-stranded DNase activity, Science 360 (2018) 436–439.
[37] J.S. Gootenberg, O.O. Abudayyeh, M.J. Kellner, J. Joung, J.J. Collins, F. Zhang, Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6, Science 360 (2018) 439–444.
[38] R. Peng, G. Lin, J. Li, Potential pitfalls of CRISPR/Cas9-mediated genome editing, FEBS J. 283 (2016) 1218–1231.
[39] D. Bohaciakova, T. Renzova, V. Fedorova, M. Barak, M. Kunova Bosakova, A. Hampl, et al., An efficient method for generation of knockout human embryonic stem cells using CRISPR/Cas9 System, Stem Cells Dev. 26 (2017) 1521–1527.
[40] X. Guo, X.J. Li, Targeted genome editing in primate embryos, Cell Res. 25 (2015) 767–768.
[41] Z. Tu, W. Yang, S. Yan, A. Yin, J. Gao, X. Liu, et al., Promoting Cas9 degradation reduces mosaic mutations in non-human primate embryos, Sci. Rep. 7 (2017), 42081.
[42] K.A. Schaefer, W.H. Wu, D.F. Colgan, S.H. Tsang, A.G. Bassuk, V.B. Mahajan, Unexpected mutations after CRISPR–Cas9 editing in vivo, Nat. Methods 14 (2017) 547–548.
[43] J. Zischewski, R. Fischer, L. Bortesi, Detection of on-target and off-target mutations generated by CRISPR/Cas9 and other sequence-specific nucleases, Biotechnol. Adv. 35 (2017) 95–104.
[44] A. Jelenkovic, R. Sund, Y.M. Hur, Y. Yokoyama, J.V. Hjelmborg, S. Moller, et al., Genetic and environmental influences on height from infancy to early adulthood: An individual-based pooled analysis of 45 twin cohorts, Sci. Rep. 6 (2016), 28496.
[45] S. Wu, S. Powers, W. Zhu, Y.A. Hannun, Substantial contribution of extrinsic risk factors to cancer development, Nature 529 (2016) 43–47.
[46] Code of Federal Regulations: Food and Drugs, 21 C.F.R. (*scattered*), 2017.
[47] United States Code: Regulation of Biological Products, 42 U.S. Code Sect 2622017.
[48] Such guidance is inapplicable to cells, tissues, and cellular- and tissue-based products regulated under United States Code: Regulations to Control Communicable Diseases, 42 U.S. Code Sect 2642017.
[49] J. Kimmelman, Ethics of cancer gene transfer clinical research, Methods Mol. Biol. 1317 (2015) 263–285.
[50] Y.D. Tang, J.T. Liu, T.Y. Wang, M.X. Sun, Z.J. Tian, X.H.C. R.I.S.P.R. Cai, Cas9-mediated multiple single guide RNAs potently abrogate pseudorabies virus replication, Arch. Virol. 162 (2017) 3881–3886.
[51] S.L. Shipman, J. Nivala, J.D. Macklis, G.M. Church, CRISPR–Cas encoding of a digital movie into the genomes of a population of living bacteria, Nature 547 (2017) 345–349.
[52] S.J. Gratz, A.M. Cummings, J.N. Nguyen, D.C. Hamm, L.K. Donohue, M.M. Harrison, et al., Genome engineering of *Drosophila* with the CRISPR RNA-guided Cas9 nuclease, Genetics 194 (2013) 1029–1035.
[53] S. Dong, J. Lin, N.L. Held, R.J. Clem, A.L. Passarelli, A.W. Franz, Heritable CRISPR/Cas9-mediated genome editing in the yellow fever mosquito, *Aedes aegypti*, PLoS One 10 (2015), e0122353.
[54] J.E. DiCarlo, J.E. Norville, P. Mali, X. Rios, J. Aach, G.M. Church, Genome engineering in *Saccharomyces cerevisiae* using CRISPR–Cas systems, Nucleic Acids Res. 41 (2013) 4336–4343.
[55] Y. Kraus, A. Aman, U. Technau, G. Genikhovich, Pre-bilaterian origin of the blastoporal axial organizer, Nat. Commun. 7 (2016), 11694.

[56] A.E. Friedland, Y.B. Tzur, K.M. Esvelt, M.P. Colaiacovo, G.M. Church, J.A. Calarco, Heritable genome editing in *C. elegans* via a CRISPR–Cas9 system, Nat. Methods 10 (2013) 741–743.
[57] U. Irion, J. Krauss, C. Nusslein-Volhard, Precise and efficient genome editing in zebrafish using the CRISPR/Cas9 system, Development 141 (2014) 4827–4830.
[58] Z. Feng, B. Zhang, W. Ding, X. Liu, D.L. Yang, P. Wei, et al., Efficient genome editing in plants using a CRISPR/Cas system, Cell Res. 23 (2013) 1229–1232.
[59] T. Hai, F. Teng, R. Guo, W. Li, Q. Zhou, One-step generation of knockout pigs by zygote injection of CRISPR/Cas system, Cell Res. 24 (2014) 372–375.
[60] M. Ikeda, S. Matsuyama, S. Akagi, K. Ohkoshi, S. Nakamura, S. Minabe, et al., Correction of a disease mutation using CRISPR/Cas9-assisted genome editing in Japanese black cattle, Sci. Rep. 7 (2017), 17827.
[61] E. Zuo, Y.J. Cai, K. Li, Y. Wei, B.A. Wang, Y. Sun, et al., One-step generation of complete gene knockout mice and monkeys by CRISPR/Cas9-mediated gene editing with multiple sgRNAs, Cell Res. 27 (2017) 933–945.
[62] H. Ma, N. Marti-Gutierrez, S.W. Park, J. Wu, Y. Lee, K. Suzuki, et al., Correction of a pathogenic gene mutation in human embryos, Nature 548 (2017) 413–419.
[63] X. Kang, W. He, Y. Huang, Q. Yu, Y. Chen, X. Gao, et al., Introducing precise genetic modifications into human 3PN embryos by CRISPR/Cas-mediated genome editing, J. Assist. Reprod. Genet. 33 (2016) 581–588.
[64] T. Gaj, C.A. Gersbach, C.F. Barbas III, ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering, Trends Biotechnol. 31 (2013) 397–405.
[65] T. Nakamura, A.R. Gehrke, J. Lemberg, J. Szymaszek, N.H. Shubin, Digits and fin rays share common developmental histories, Nature 537 (2016) 225–228.
[66] J.J. Russell, J.A. Theriot, P. Sood, W.F. Marshall, L.F. Landweber, L. Fritz-Laylin, et al., Non-model model organisms, BMC Biol. 15 (2017) 55.
[67] W. Shang, F. Wang, G. Fan, H. Wang, Key elements for designing and performing a CRISPR/Cas9-based genetic screen, J. Genet. Genomics 44 (2017) 439–449.
[68] H.K. Liao, F. Hatanaka, T. Araoka, P. Reddy, M.Z. Wu, Y. Sui, et al., In vivo target gene activation via CRISPR/Cas9-Mmdiated trans-epigenetic modulation, Cell 171 (2017) (1495-507.e15).
[69] M. Yang, L. Zhang, J. Stevens, G. Gibson, CRISPR/Cas9 mediated generation of stable chondrocyte cell lines with targeted gene knockouts; analysis of an aggrecan knockout cell line, Bone 69 (2014) 118–125.
[70] X.J. Lu, Y.Y. Xiang, X.J. Li, CRISPR screen: a high-throughput approach for cancer genetic research, Clin. Genet. 88 (2015) 32–33.
[71] E. Charpentier, H. Richter, J. van der Oost, M.F. White, Biogenesis pathways of RNA guides in archaeal and bacterial CRISPR–Cas adaptive immunity, FEMS Microbiol. Rev. 39 (2015) 428–441.
[72] T.S. Klann, J.B. Black, M. Chellappan, A. Safi, L. Song, I.B. Hilton, et al., CRISPR–Cas9 epigenome editing enables high-throughput screening for functional regulatory elements in the human genome, Nat. Biotechnol. 35 (2017) 561–568.
[73] A. Mazo-Vargas, C. Concha, L. Livraghi, D. Massardo, R.W. R. Wallbank, L. Zhang, et al., Macroevolutionary shifts of WntA function potentiate butterfly wing-pattern diversity, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) 10701–10706.
[74] P. Atanes, I. Ruz-Maldonado, R. Hawkes, B. Liu, S.J. Persaud, S. Amisten, Identifying signalling pathways regulated by GPRC5B in beta-cells by CRISPR–Cas9-mediated genome editing, Cell. Physiol. Biochem. 45 (2018) 656–666.
[75] S. Chi, A. Weiss, H.A. Wang, CRISPR-based toolbox for studying T cell signal transduction, Biomed. Res. Int. 2016 (2016), 5052369.
[76] C. Kasap, O. Elemento, T.M. Kapoor, DrugTargetSeqR: a genomics- and CRISPR–Cas9-based method to analyze drug targets, Nat. Chem. Biol. 10 (2014) 626–628.
[77] B.E. Housden, A.J. Valvezan, C. Kelley, R. Sopko, Y. Hu, C. Roesel, et al., Identification of potential drug targets for tuberous sclerosis complex by synthetic screens combining CRISPR-based knockouts with RNAi, Sci. Signal. 8 (2015) rs9.
[78] I. Hyun, A. Wilkerson, J. Johnston, Embryology policy: revisit the 14-day rule, Nature 533 (7602) (2016) 169–171.
[79] Human embryo culture: discussions concerning the statutory time limit for maintaining human embryos in culture in light of some recent scientific developments. Nuffield Council on Bioethics, 2017 [cited 2018 May 29]. Available from: http://nuffieldbioethics.org/wp-content/uploads/Human-Embryo-Culture-web-FINAL.pdf.
[80] R.A. Charo, The hunting of the snark: the moral status of embryos, right-to-lifers, and Third World women, Stanf. Law Pol. Rev. 6 (1995) 11–37.
[81] B. Wennergren, Human rights of an embryo, J. Int. Bioethique 2 (1991) 46–49.
[82] J.J. Miklavcic, P. Flaman, Personhood status of the human zygote, embryo, fetus, Linacre Q. 84 (2017) 130–144.
[83] J.W. McDonald, X.Z. Liu, Y. Qu, S. Liu, S.K. Mickey, D. Turetsky, et al., Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord, Nat. Med. 5 (1999) 1410–1412.
[84] L.M. Bjorklund, R. Sanchez-Pernaute, S. Chung, T. Andersson, I.Y. Chen, K.S. McNaught, et al., Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinson rat model, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 2344–2349.
[85] V.F. Hamrahi, J. Goverman, W. Jung, J.C. Wu, A.J. Fischman, R.G. Tompkins, et al., In vivo molecular imaging of murine embryonic stem cells delivered to a burn wound surface via Integra(R) scaffolding, J. Burn Care Res. 33 (2012) e49–e54.
[86] D.K. Singla, A. Ahmed, R. Singla, B. Yan, Embryonic stem cells improve cardiac function in doxorubicin-induced cardiomyopathy mediated through multiple mechanisms, Cell Transplant. 21 (2012) 1919–1930.
[87] F.P. Busardo, M. Gulino, S. Napoletano, S. Zaami, P. Frati, The evolution of legislation in the field of medically assisted reproduction and embryo stem cell research in European union members, Biomed. Res. Int. 2014 (2014), 307160.
[88] R. Dajani, Jordan's stem-cell law can guide the Middle East, Nature 510 (2014) 189.
[89] R.T. Manguso, H.W. Pope, M.D. Zimmer, F.D. Brown, K.B. Yates, B.C. Miller, et al., In vivo CRISPR screening identifies Ptpn2 as a cancer immunotherapy target, Nature 547 (2017) 413–418.
[90] J. Drost, R. van Boxtel, F. Blokzijl, T. Mizutani, N. Sasaki, V. Sasselli, et al., Use of CRISPR-modified human stem cell organoids to study the origin of mutational signatures in cancer, Science 358 (2017) 234–238.
[91] J. Chu, G. Galicia-Vazquez, R. Cencic, J.R. Mills, A. Katigbak, J.A. Porco Jr., et al., CRISPR-mediated drug-

target validation reveals selective pharmacological inhibition of the RNA helicase, eIF4A, Cell Rep. 15 (2016) 2340–2347.
[92] S.H. Hough, A. Ajetunmobi, L. Brody, N. Humphryes-Kirilov, E. Perello, Desktop Genetics, Perinat. Med. 13 (2016) 517–521.
[93] Z. Huang, A. Tomitaka, A. Raymond, M. Nair, Current application of CRISPR/Cas9 gene-editing technique to eradication of HIV/AIDS, Gene Ther. 24 (2017) 377–384.
[94] L.A. George, S.K. Sullivan, A. Giermasz, J.E.J. Rasko, B.J. Samelson-Jones, J. Ducore, et al., Hemophilia B gene therapy with a high-specific-activity factor IX variant, N. Engl. J. Med. 377 (2017) 2215–2227.
[95] C. Wei, F. Wang, W. Liu, W. Zhao, Y. Yang, K. Li, et al., CRISPR/Cas9 targeting of the androgen receptor suppresses the growth of LNCaP human prostate cancer cells, Mol. Med. Rep. 17 (2018) 2901–2906.
[96] T.W.Y. Wong, R.D. Cohn, Therapeutic applications of CRISPR/Cas for Duchenne muscular dystrophy, Curr. Gene Ther. 17 (2017) 301–308.
[97] T. Gaj, D.S. Ojala, F.K. Ekman, L.C. Byrne, P. Limsirichai, D.V. Schaffer, In vivo genome editing improves motor function and extends survival in a mouse model of ALS, Sci. Adv. 3 (2017) eaar3952.
[98] J. Wen, W. Tao, S. Hao, Y. Zu, Cellular function reinstitution of offspring red blood cells cloned from the sickle cell disease patient blood post CRISPR genome editing, J. Hematol. Oncol. 10 (2017) 119.
[99] G. Schwank, B.K. Koo, V. Sasselli, J.F. Dekkers, I. Heo, T. Demircan, et al., Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients, Cell Stem Cell 13 (2013) 653–658.
[100] Y. Wu, H. Zhou, X. Fan, Y. Zhang, M. Zhang, Y. Wang, et al., Correction of a genetic disease by CRISPR–Cas9-mediated gene editing in mouse spermatogonial stem cells, Cell Res. 25 (2015) 67–79.
[101] Code of Federal Regulations: Investigational New Drug, 21 C.F.R. Sec 3122017.
[102] G. Suntharalingam, M.R. Perry, S. Ward, S.J. Brett, A. Castello-Cortes, M.D. Brunner, et al., Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412, N. Engl. J. Med. 355 (2006) 1018–1028.
[103] R. Kaur, P. Sidhu, S. Singh, What failed BIA 10-2474 Phase I clinical trial? Global speculations and recommendations for future Phase I trials, J. Pharmacol. Pharmacother. 7 (2016) 120–126.
[104] B. Szikriszt, A. Poti, O. Pipek, M. Krzystanek, N. Kanu, J. Molnar, et al., A comprehensive survey of the mutagenic impact of common cancer cytotoxics, Genome Biol. 17 (2016) 99.
[105] T. Friedmann, E.C. Jonlin, King NMP, B.E. Torbett, N.A. Wivel, Y. Kaneda, et al., ASGCT and JSGT Joint position statement on human genomic editing, Mol. Ther. 23 (2015) 1282.
[106] X. Wang, Y. Niu, J. Zhou, H. Yu, Q. Kou, A. Lei, et al., Multiplex gene editing via CRISPR/Cas9 exhibits desirable muscle hypertrophy without detectable off-target effects in sheep, Sci. Rep. 6 (2016), 32271.
[107] C. Brokowski, M. Pollack, R. Pollack, Cutting eugenics out of CRISPR–Cas9, EBEM 6 (2015) 263.
[108] Buck v. Bell, 274 U.S. 2001927.
[109] J.D. Smith, K.R. Nelson, The Sterilization of Carrie Buck, New Horizon Press, Far Hills, N.J., 1989
[110] P.A. Lombardo, Three Generations, No Imbeciles: Eugenics, the Supreme Court, and Buck v. Bell, Johns Hopkins University Press, Baltimore, 2008.
[111] V.M. Gantz, N. Jasinskiene, O. Tatarenkova, A. Fazekas, V.M. Macias, E. Bier, et al., Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito *Anopheles stephensi*, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) E6736–E6743.
[112] J. Savulescu, N. Bostrom, Human Enhancement, Oxford University Press, New York, 2009.
[113] United Nations, Food, [cited 2017 Dec 23]. Available from: http://www.un.org/sustainabledevelopment/hunger/.
[114] World Health Organization, Nutrition, [cited 2017 Dec 23]. Available from: http://apps.who.int/iris/bitstream/10665/43412/1/9241594012_eng.pdf?ua=1.
[115] E. Stout, T. Klaenhammer, R. Barrangou, CRISPR–Cas technologies and applications in food bacteria, Annu. Rev. Food Sci. Technol. 8 (2017) 413–437.
[116] Y. Demirci, B. Zhang, T. Unver, CRISPR/Cas9: an RNA-guided highly precise synthetic tool for plant genome editing, J. Cell. Physiol. 233 (2018) 1844–1859.
[117] J. Zhang, H. Zhang, J.R. Botella, J.K. Zhu, Generation of new glutinous rice by CRISPR/Cas9-targeted mutagenesis of the Waxy gene in elite rice varieties, J. Integr. Plant Biol. 60 (2018) 369–375.
[118] S.R. Rajendran, Y.Y. Yau, D. Pandey, A. Kumar, CRISPR–Cas9 based genome engineering: opportunities in agri-food-nutrition and healthcare, OMICS 19 (2015) 261–275.
[119] H. Du, X. Zeng, M. Zhao, X. Cui, Q. Wang, H. Yang, et al., Efficient targeted mutagenesis in soybean by TALENs and CRISPR/Cas9, J. Biotechnol. 217 (2016) 90–97.
[120] S. Svitashev, J.K. Young, C. Schwartz, H. Gao, S.C. Falco, Cigan A.M. Targeted Mutagenesis, Precise gene editing, and site-specific gene insertion in maize using Cas9 and guide RNA, Plant Physiol. 169 (2015) 931–945.
[121] K. Brinegar, K.Y. A, S. Choi, E. Vallillo, G.U. Ruiz-Esparza, A.M. Prabhakar, et al., The commercialization of genome-editing technologies, Crit. Rev. Biotechnol. 37 (2017) 924–932.
[122] F. Georges, H. Ray, Genome editing of crops: a renewed opportunity for food security, GM Crops Food. 8 (2017) 1–12.
[123] S. Gonen, J. Jenko, G. Gorjanc, A.J. Mileham, C.B. Whitelaw, J.M. Hickey, Potential of gene drives with genome editing to increase genetic gain in livestock breeding programs, Genet. Sel. Evol. 49 (2017) 3.
[124] V.M. Gantz, E. Bier, Genome editing. The mutagenic chain reaction: a method for converting heterozygous to homozygous mutations, Science 348 (2015) 442–444.
[125] A. Hammond, R. Galizi, K. Kyrou, A. Simoni, C. Siniscalchi, D. Katsanos, et al., A CRISPR–Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*, Nat. Biotechnol. 34 (2016) 78–83.
[126] National Academies of Sciences Engineering and Medicine (U.S.), Committee on Gene Drive Research in Non-Human Organisms: Recommendations for Responsible Conduct. Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research With Public VALUes, The National Academies Press, Washington, D.C., 2016
[127] N. Windbichler, M. Menichelli, P.A. Papathanos, S.B. Thyme, H. Li, U.Y. Ulge, et al., A synthetic homing endonuclease-based gene drive system in the human malaria mosquito, Nature 473 (2011) 212–215.
[128] P.A. Eckhoff, E.A. Wenger, H.C. Godfray, A. Burt, Impact of mosquito gene drive on malaria elimination in a computational model with explicit spatial and temporal dynamics, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) (E255-E64).

[129] S. James, F.H. Collins, P.A. Welkhoff, C. Emerson, H.C.J. Godfray, M. Gottlieb, et al., Pathway to deployment of gene drive mosquitoes as a potential biocontrol tool for elimination of malaria in sub-Saharan Africa: recommendations of a scientific working group, Am. J. Trop. Med. Hyg. 98 (2018) 1–49.
[130] N.L. Achee, F. Gould, T.A. Perkins, R.C. Reiner Jr., A.C. Morrison, S.A. Ritchie, et al., A critical assessment of vector control for dengue prevention, PLoS Negl. Trop. Dis. 9 (2015), e0003655.
[131] K.M. Esvelt, A.L. Smidler, F. Catteruccia, G.M. Church, Concerning RNA-guided gene drives for the alteration of wild populations, elife 3 (2014).
[132] S.H. Sokolow, C.L. Wood, I.J. Jones, K.D. Lafferty, A.M. Kuris, M.H. Hsieh, et al., To reduce the global burden of human schistosomiasis, use 'old fashioned' snail control, Trends Parasitol. 34 (2018) 23–40.
[133] D. Moro, M. Byrne, M. Kennedy, S. Campbell, M. Tizard, Identifying knowledge gaps for gene drive research to control invasive animal species: the next CRISPR step, Glob. Ecol. Conserv. 13 (2018), e00363.
[134] J.E. DiCarlo, A. Chavez, S.L. Dietz, K.M. Esvelt, G.M. Church, Safeguarding CRISPR–Cas9 gene drives in yeast, Nat. Biotechnol. 33 (2015) 1250–1255.
[135] E. Roggenkamp, R.M. Giersch, M.N. Schrock, E. Turnquist, M. Halloran, G.C. Finnigan, Tuning CRISPR–Cas9 gene drives in *Saccharomyces cerevisiae*, G3 (Bethesda) 8 (2018) 999–1018.
[136] E.M. Basgall, S.C. Goetting, M.E. Goeckel, R.M. Giersch, E. Roggenkamp, M.N. Schrock, et al., Gene drive inhibition by the anti-CRISPR proteins AcrIIA2 and AcrIIA4 in *Saccharomyces cerevisiae*, Microbiology 164 (2018) 464–474.
[137] M.R. Vella, C.E. Gunning, A.L. Lloyd, F. Gould, Evaluating strategies for reversing CRISPR–Cas9 gene drives, Sci. Rep. 7 (2017), 11038.
[138] U.S. CONST. amend. 1791, 1.
[139] E.K. White, Killing U.S. slowly: curing the epidemic rise of cancer drug prices, Food Drug Law J. 72 (2017) 189–224.
[140] L. Cong, F.A. Ran, D. Cox, S. Lin, R. Barretto, N. Habib, et al., Multiplex genome engineering using CRISPR/Cas systems, Science 339 (2013) 819–823.
[141] S. Chen, N.E. Sanjana, K. Zheng, O. Shalem, K. Lee, X. Shi, et al., Genome-wide CRISPR screen in a mouse model of tumor growth and metastasis, Cell 160 (2015) 1246–1260.
[142] H. Wang, H. Yang, C.S. Shivalila, M.M. Dawlaty, A.W. Cheng, F. Zhang, et al., One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering, Cell 153 (2013) 910–918.
[143] F.A. Ran, L. Cong, W.X. Yan, D.A. Scott, J.S. Gootenberg, A.J. Kriz, et al., In vivo genome editing using *Staphylococcus aureus* Cas9, Nature 520 (2015) 186–191.
[144] I.M. Slaymaker, L. Gao, B. Zetsche, D.A. Scott, W.X. Yan, F. Zhang, Rationally engineered Cas9 nucleases with improved specificity, Science 351 (2016) 84–88.
[145] R.D. Chow, C.D. Guzman, G. Wang, F. Schmidt, M.W. Youngblood, L. Ye, et al., AAV-mediated direct in vivo CRISPR screen identifies functional suppressors in glioblastoma, Nat. Neurosci. 20 (2017) 1329–1341.
[146] J. Doudna, A. Mason, Federal Funding for Basic Research Led to the Gene-Editing Revolution. Don't Cut It, Vox, 2017.
[147] M.A. Moreno-Mateos, J.P. Fernandez, R. Rouet, C.E. Vejnar, M.A. Lane, E. Mis, et al., CRISPR–Cpf1 mediates efficient homology-directed repair and temperature-controlled genome editing, Nat. Commun. 8 (2017) 2024.
[148] D. Burstein, L.B. Harrington, S.C. Strutt, A.J. Probst, K. Anantharaman, B.C. Thomas, et al., New CRISPR–Cas systems from uncultivated microbes, Nature 542 (2017) 237–241.
[149] S.H. Sternberg, B. LaFrance, M. Kaplan, J.A. Doudna, Conformational control of DNA target cleavage by CRISPR–Cas9, Nature 527 (2015) 110–113.
[150] K. Schumann, S. Lin, E. Boyer, D.R. Simeonov, M. Subramaniam, R.E. Gate, et al., Generation of knock-in primary human T cells using Cas9 ribonucleoproteins, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 10437–10442.
[151] M.R. O'Connell, B.L. Oakes, S.H. Sternberg, A. East-Seletsky, M. Kaplan, J.A. Doudna, Programmable RNA recognition and cleavage by CRISPR/Cas9, Nature 516 (2014) 263–266.
[152] J.A. Greene, W.V. Padula, Targeting unconscionable prescription-drug prices—Maryland's Anti-Price-Gouging Law, N. Engl. J. Med. 377 (2017) 101–103.
[153] E. Marshall, Gene therapy death prompts review of adenovirus vector, Science 286 (1999) 2244–2245.
[154] K.M. Frank, D.K. Hogarth, J.L. Miller, S. Mandal, P.J. Mease, R.J. Samulski, et al., Investigation of the cause of death in a gene-therapy trial, N. Engl. J. Med. 361 (2009) 161–169.
[155] C.E. Dunbar, K.A. High, J.K. Joung, D.B. Kohn, K. Ozawa, M. Sadelain, Gene therapy comes of age, Science 359 (2018).
[156] Code of Federal Regulations: Food and Drugs, Institutional Review Boards, 21 C.F.R. 562017.
[157] Code of Federal Regulations: Protection of Human Subjects, 45 C.F.R. Sect 462017.
[158] Code of Federal Regulations: Food and Drugs, Protection of Human Subjects, 21 C.F.R. 502017.
[159] Code of Federal Regulations: Food and Drugs, Investigational New Drug Application, 21 C.F.R. 3122017.
[160] Code of Federal Regulations: Food and Drugs, Investigational Device, 21 C.F.R. 8122017.
[161] NIH to launch genome editing research program, https://www.nih.gov/news-events/news-releases/nih-launch-genome-editing-research-program.
[162] Commission on Genetic Modification and the Health Council of the Netherlands, Editing human DNA: moral and social implications of germline genetic modification, 2017, Accessed 26 May 2018.
[163] The Academy of Medical Sciences' response to the Nuffield Council on Bioethics 'Genome editing and human reproduction', Acad. Med. Sci. (2017) [[press release] [cited 2018 May 26]. Available from: https://acmedsci.ac.uk/file-download/89659337].
[164] Document on bioethics and gene editing in humans, [cited 2018 May 26]. Available from: Bioethics and Law Observatory of the University of Barcelona, http://www.publicacions.ub.edu/refs/observatoriBioEticaDret/documents/08543.pdf 2016.
[165] G. Church, Compelling reasons for repairing human germlines, N. Engl. J. Med. 377 (2017) 1909–1911.
[166] Geneva Convention, Protocol for the prohibition of the use in war of asphyxiating, poisonous or other gases, and of bacteriological methods of warfare, http://avalon.law.yale.edu/20th_century/geneva01.asp 1925, Accessed date: 20 December 2017.

[167] United Nations, Convention on the prohibition of the development, production and stockpiling of bacteriological (biological) and toxin weapons and on their destruction, http://avalon.law.yale.edu/20th_century/bact.asp 1975, Accessed date: 20 December 2017.
[168] M. Enserink, Interested in responsible genome editing? Join the new club, Science (2018), https://doi.org/10.1126/science.aat7183.
[169] M. Lluis, M. Jennifer, H. François, A. Marion, J. Pierre, B. Bernard, S.M. Cyril, C. Hervé, ARRIGE arrives: toward the responsible use of genome editing, CRISPR J. 1 (2) (2018) 128–129.
[170] S. Jasanoff, J.B. Hurlbut, A global observatory for gene editing, Nature 555 (2018) 435–437.