CRISPR germline engineering—the community speaks

Katrine S Bosley, Michael Botchan, Annelien L Bredenoord, Dana Carroll, R Alta Charo, Emmanuelle Charpentier, Ron Cohen, Jacob Corn, Jennifer Doudna, Guoping Feng, Henry T Greely, Rosario Isasi, Weihzi Ji, Jin-Soo Kim, Bartha Knoppers, Edward Lanphier, Jinsong Li, Robin Lovell-Badge, G Steven Martin, Jonathan Moreno, Luigi Naldini, Martin Pera, Anthony CF Perry, J Craig Venter, Feng Zhang & Qi Zhou

Nature Biotechnology **asks selected members of the international community to comment on the ethical issues raised by the prospect of CRISPR-Cas9 engineering of the human germline.**

With the first papers appearing in the literature that describe CRISPR-Cas9 engineering of human reproductive cells, are we at a new Asilomar moment? In a letter to *Science* in March entitled "A prudent path forward for genomic engineering and germline gene modification," 18 signers indicated "A framework for open discourse on the use of CRISPR-Cas9 technology to manipulate the human genome is urgently needed." They wrote of "unparalleled potential for modifying human and nonhuman genomes," to cure genetic diseases in humans and to "reshape the biosphere." But they warned of consequent "unknown risks to human health and well-being."

Nature Biotechnology contacted 50 researchers, ethicists and business leaders in the global community to comment on ethical issues raised by CRISPR engineering of the human germline. *Nature Biotechnology* received responses from 26 of those contacted; because of space constraints, only an edited sample of all the responses received are presented below. Readers are directed to **Supplementary Comments** for the unedited responses received, which will give an idea of the breadth of agreement among different respondents on different issues.

With the current pace of advances in the use of gene editing technology, IVF and germ stem cell research, to what extent do you think germline engineering is inevitable?

Alta Charo: I do not think it is inevitable because many of the reasons one might imagine using it in the future might also suggest the use of easier technologies involving selection among gametes and embryos free of the destructive trait of interest.

Robin Lovell-Badge: It is inevitable and will be carried out somewhere, given that it is not illegal in many countries. But it is difficult to predict when, or for what purpose.

Annelien Bredenoord: I prefer not to use the word 'inevitable' because in the end it would be a consequence of human decision-making. I am inclined to say that inheritable genetic modification is on the horizon, but perhaps the first application of germline modification would be mitochondrial donation (also known as mitochondrial gene transfer or mitochondrial gene therapy), which does not involve gene-editing techniques. Recently, the UK Parliament legalized this technique

Katrine S. Bosley is at Editas Medicine, Cambridge, Massachusetts, USA; Michael Botchan is in the Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, California, USA; Annelien Bredenoord is in the Department of Medical Humanities, University Medical Center, Utrecht, the Netherlands; Dana Carroll is in the Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, Utah, USA; R. Alta Charo is at the School of Law, and Department of Medical History and Bioethics, University of Wisconsin School of Medicine & Public Health, Madison, Wisconsin, USA; Emmanuelle Charpentier is in the Department of Regulation in Infection Biology, Helmholtz Centre for Infection Research, Braunschweig, Germany; Ron Cohen is at Acorda Therapeutics, Ardsley, New York, USA; Jacob Corn is at the Innovative Genomics Initiative, Berkeley, California, USA; Jennifer Doudna is in Department of Molecular & Cell Biology and Chemistry, University of California, Berkeley, Berkeley, California, USA; Guoping Feng is in the Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard University, Cambridge, Massachusetts, USA; Henry T (Hank) Greely is at Stanford Law School, Stanford, California, USA; Rosario Isasi is in the Department of Human Genetics, McGill University, Montreal, Quebec, Canada; Weihzi Ji is at the Kunming Biomed International and National Engineering Research Center of Biomedicine and Animal Science, Kunming, China; Jin-Soo Kim is at the Center for Genome Engineering, Institute for Basic Science and Department of Chemistry, Seoul National University, Seoul, Korea; Bartha Knoppers is in the Department of Human Genetics, McGill University, Montreal, Quebec, Canada; Edward Lanphier is at Sangamo Biosciences, Richmond, California, USA; Jinsong Li is at the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China; Robin Lovell-Badge is at The Francis Crick Institute, London, UK; G. Steven Martin is in the Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, California, USA; Jonathan Moreno is at the University of Pennsylvania, Philadelphia, Pennsylvania, USA; Luigi Naldini is at the San Raffaele Telethon Institute for Gene Therapy, Milan, Italy; Martin Pera is in the Department of Anatomy and Neuroscience, University of Melbourne, Melbourne, Australia; Anthony Perry is in the Department of Biology and Biochemistry, University of Bath, Bath, UK; J. Craig Venter is at the J. Craig Venter Institute in La Jolla, California, USA; Feng Zhang is at the Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA; Qi Zhou is at the State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China.

aimed at preventing the transmission of mitochondrial DNA mutations from mother to child.

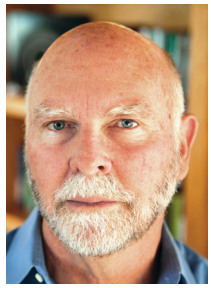
Katrine Bosley: From a technical standpoint, I think most scientists think that this would be relatively straightforward, but technical feasibility is never the only consideration in doing experiments. For example, every day, we also think about safety of experiments (for people working in and around labs, for the local community, etc.), about environmental concerns (how we manage chemicals, radiation, etc.), and, of course, about ethics in many different dimensions (in animal research, in informed consent of human subjects, in design of clinical trials, etc.). There's a robust framework for all of these considerations—laws, regulations, policies and general good practices—that has been developed over many years and is part of how we train scientists and [of] daily working practice. I think that this will be the case for human germline engineering as well, particularly given that the societal and ethical issues surrounding it are broad and profound. Human germline engineering isn't a new concept, but we haven't had to think deeply about its management or regulation until now because it was pretty theoretical until now. As is often the case, a technical breakthrough is forcing us to confront a complicated question fast. But I have confidence we will address it carefully and thoughtfully—the fact that this dialog is emerging so early in the life of this technology shows that the scientific community sees the implications and sees the need for and the importance of broadening the dialog beyond the people working in the field and indeed beyond scientists and clinicians. Everyone has a stake in getting this right, and there are a lot of different perspectives around the table that need to be part of the discussion. I think we have a responsibility both to find the right way to realize the potential of this powerful technology and also to do it in a way that is highly ethical.

Katrine Bosley

Tony Perry: Human germline genome engineering is probably inevitable, although it's unclear how quickly it will come about. One can simplify it to three issues: the tools, the goals and whether the tools can achieve the goals. We will likely soon have the tools. The goals are a major focus of the ethical debate that will determine when and if human germline genome engineering is implemented. There may be insuperable barriers to the tools achieving complex goals like higher IQ compared with, say, the modification of a highly penetrant mutation to prevent a disease.

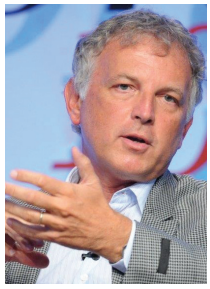
Ron Cohen: It is inevitable. No way to stop it, only to regulate it as best as possible.

J. Craig Venter: I think that human germline engineering is inevitable, and there will be basically no effective way to regulate or control the use of gene editing technology in human reproduction. Our species will stop at nothing to try to improve positive perceived traits and to eliminate disease risk or to remove perceived negative traits from the future offspring, particularly by those with the means or access to editing and reproductive technology. The question is when, not if.



J. Craig Venter

What are the major outstanding technical barriers to achieving germline alteration for human clinical application?



Luigi Naldini

Luigi Naldini: Whereas gene disruption is easily within the reach of current technologies, gene editing is not. Gene editing (which would be required for *in situ* correction of a mutation or editing of a risk- or disease-causing allelic variant) relies on gene targeting (by artificial endonucleases) and homologous recombination using an exogenous template. Current methods for gene editing are inefficient in primary cells and require selection of a small fraction of the treated cells bearing the desired edit. This is not easily applicable to germline engineering, especially in humans. First, one would have to treat a very large number of embryos to have a reasonable chance to generate some edited cells and there is no obvious (to me) strategy to identify and select those (even fewer) treated embryos carrying the desired edit in most if not all inner mass cells, unless by forced selection through a genetic switch built-in within the template. The majority of treated embryos would carry a targeted, possibly disrupted, allele and, in the absence of forced selection (or a rarely occurring situation in which gene correction *per se* endows ES cells with a selective advantage), the few embryos carrying edited cells would be chimeras. Second, current embryo screening and implantation strategies would not address the occurrence and/or extent of chimeras and seem hardly compatible with the expected efficiency. Gene editing combined with (exogenous) genetic selection would entail a more substantial genetic modification of the germline (incorporation of exogenous selector) similar to the GMOs currently used in agriculture or transgenic animal models and raise even more concerns on acceptability and potential risks. Current hurdles toward achieving efficient editing in primary cell types are efficient delivery of the gene targeting machinery, tolerance and permissiveness and/or proficiency of the treated cell to homologous recombination, selection of the desired edit, possibly epigenetic scar at the targeted gene altering expression features.

Jinsong Li: Of the two main strategies for germline modification—transfecting CRISPR-Cas9 into zygotes or injecting CRISPR-Cas9 into germ stem cells (which then produce gametes carrying corrected genes)—engineering of germ stem cells has more promise. In the former method, not all resulting pups carry the desired genotype and there are sufficient off-target effects to be a concern; the latter method allows gametes to be screened for presence and fidelity of the modification before creation of the zygote. There are at least three outstanding technical barriers that need to be solved before germ stem cell–mediated gene therapy can be taken into humans. Taking spermatogenesis stem cells (SSCs) as an example, these are: how to achieve efficient derivation of stem cell lines in humans; second, will it be possible to obtain mature sperm from cultured SSCs; and third, will it be possible to achieve efficient genetic modification of human SSCs? In my mind, there is still a long-way to go to use CRISPR-Cas9 in germ cells to correct human genetic disease.

Jinsong Li

Jin-Soo Kim: Before moving to germline editing, researchers need to develop, first, methods to suppress error-prone, NHEJ and to enhance the efficiency of HDR in germ cells; second, improvements in the methods for profiling genome-wide off-target sites (e.g., Digenome-seq, GUIDE-seq) to reduce or avoid false-positive and false-negative sites; and third, sensitive methods to measure off-target mutation frequencies. Current sequencing platforms often cannot detect off-target mutations that are induced at frequencies below 0.1%.

Feng Zhang: There are challenges on both the technical and biological fronts. Technologically, we don't know how specific the current generation of genome-editing tools is. Do these tools result in any other changes in the genome? Do they affect the cell in other undesirable ways, such as altering the epigenetic state of the genome and lead to other lasting consequences? Biologically, we still know very little about how changes in the genome may affect biological function. With the exception of a small number of mutations that are known to cause diseases, we are unable to predict the biological consequence of any specific genetic change in a cell or organism.

Guoping Feng. One of the major issues is off-target effects. A second issue is the potential mosaicism from editing after the single-cell stage. A third issue is the low efficiency of HDR for correcting genetic defects. However, these are technical barriers that will be solved in the near future. In fact, progress has been made in each of the areas, such as the use of double nickases to reduce off-target effects, using Cas9 or nickase protein instead of mRNA for faster action, and the suppression of genetic programs to increase HDR efficiency.

Guoping Feng

Edward Lanphier: Achievement of a high degree of specificity that is essential for therapeutic use, particularly for the CRISPR-Cas9 system, which is the least specific of all of the current methods of genome editing (ZFNs and TALENs), and efficient delivery protocols to lessen the possibility of chimerism of the resulting organism are the major outstanding technical barriers to achieving germline alteration for human clinical application.

What are the individual health risks associated with germline engineering and what are the potential individual benefits?

Hank Greely: The anticipated individual health risks are off-target effects and genetic chimerism. In addition, there are also unanticipated effects of on-target changes. And it may be that the process of intervention in gametes, gamete precursors, zygotes, etc., would also have some unanticipated bad effects. The potential individual benefits are trickier, I think. In only a few cases would there be medical benefits (in terms of avoiding genetic disease) that could not be obtained through preimplantation genetic diagnosis or through prenatal testing and (when wanted) abortion. The advantage that your descendants wouldn't have to use preimplantation genetic diagnosis seems pretty small to me. People who are homozygous for dominant diseases—a couple that both have the same autosomal recessive disease—may add a few more candidates for the approach, but not many more. In terms of enhancement, we're so far from knowing and understanding 'enhancing' genes, at this point the individual benefits are asymptotic to zero.

Lovell-Badge: Of course, any justification for attempting gene editing in humans must balance risk and benefit, where clinical need is the most important. Experiments in mice suggest that most gene editing experiments have not led to noticeable effects apart from those expected from targeting the gene in question. However, subtle problems will be missed, as will problems causing early embryo lethality. And mice are not humans. Although off-target effects may be rare, whether they are serious or not is going to be hard to predict without doing the 'human experiment'. Second, genetic mosaicism could be a problem depending on the gene being edited. In some cases where gene mutations in mice have been studied in mosaics or in chimeras (where two embryos are joined together), the resulting phenotype is worse than when the gene is mutated in all cells. However, generally one expects a milder version of the phenotype. Unanticipated effects of the on-target changes could occur. If there were insufficient knowledge about the gene and how it works, the change being engineered might in some cases lead to, for example, new protein-protein interactions that compromise the function of the second protein. The potential individual benefits will depend on who you are talking about: a child who would otherwise have been born with a defect, or the parent whose ego has run amok and wants some improvement in his/her child?

Jennifer Doudna, Dana Carroll, G. Steven Martin & Michael Botchan: We would list at least five risks. First, some applications would be confounded by on-target mutagenesis by NHEJ. For example, one could unintentionally convert sickle cell disease into beta thalassemia. Second, although the likelihood of off-target effects can be minimized, there is still the possibility that an essential gene could be mutated. If the individual was already heterozygous for a mutation in such a gene, this would give them two mutant alleles. Some genes are haploinsufficient, so a single mutant allele would affect them. Genes on the X chromosome are present in a single copy in males and are expressed from only one parental chromosome in cells of females, so mutations there represent a greater risk. Third, if the 'edited' individual is chimeric for the intended correction, they may still have diseased cells in critical tissues. Fourth, the genetic background in which the disease mutation exists may at some level be adapted to carrying that mutation, and correcting the gene back to 'wild type' could have unanticipated consequences in that background. We would classify this as a tertiary concern because it seems very unlikely to have significant consequences. Finally, it will be hard to predict and assess unintended long-term consequences of germline editing, such as effects that only occur later in life and result from the specific genetic background of an individual.

Jennifer Doudna

Ron Cohen

Cohen: Mostly speculative at this point, though one can predict on the basis of historical precedent with other new technologies (e.g., Jesse Gelsinger [see timeline] that off-target and unintended effects will almost certainly occur.

Tony Perry

Perry: The risks depend on the targeted sequence; some sequences may enable extremely high specificity whereas others don't. Some may have serious off-target consequences if they do occur, whereas others may not have overt consequences. Another issue is unanticipated effects of on-target changes; introducing an improving genome modification may not always be without attendant disadvantages. For example, with heterozygous carriers of the HbS single-nucleotide polymorphism for sickle cell disease, you eliminate sickle cell disease but increase the risk of contracting malaria. Benefits in general include eliminating many of the 3,000 or so single-gene heritable disease traits. In my mind, chimerism is a lower technical risk, firstly because the system is (already) so efficient, secondly because it would be highly prescriptive leading to identical end points, and thirdly because it will likely be of altered and non-altered genomes, so the person would be no worse off than they would otherwise have been.

Emmanuelle Charpentier: Besides a very significant number of ethical questions to be addressed, safety concerns are probably the most pressing consideration. During the recent debate and approval of legislation allowing mitochondrial replacement approaches for IVF in the UK—which leads to circumstances in which an embryo would receive genetic material from three different individuals—the concept of chimerism having a negative impact on the health and fitness of respective offspring was dismissed for humans. Potential benefits are related to the gene correction of severe genetic disease allowing kids a normal life.

What are the societal risks of germline engineering and what are the potential benefits?

Jonathan Moreno

Jonathan Moreno: Perhaps the obvious health benefits for future persons are evident, as well as possible savings for healthcare systems for chronic conditions and disabling conditions (although presumably everyone will always die of something so those savings might be short-term). On the other hand, population biologists suggested 40 years ago that it might be advisable to establish a bank of traits that have been screened out of populations, just in case they need to be reintroduced into the gene pool. Although they were talking about the unintended consequences of traditional screening for carriers of such conditions as sickle cell anemia and Tay-Sachs, that idea has renewed resonance now. There is also the prospect of 'consumer eugenics'—eugenics driven by parental choice rather than by state order, which would have similar results to traditional eugenics, such as a multitiered social system based on certain enhancements. In truly far-out scenarios, some states might wish to produce generations of super-charged individuals as potential warfighters. I'm thinking of *The Boys From Brazil*.

Naldini: The main current societal risk is the backlash from an exaggerated but potentially pervasive view that gene-editing technologies will lead to science-fiction scenarios in which humans are bred upon design leading to a whole array of unanticipated effects (see also Anthony Perry comment in **Supplementary Comments**). Even if these are unrealistic scenarios, they may generate fear, distrust [of] scientists and overcaution on the use of the current technologies, which may inhibit their full exploitation for less problematic and more fruitful applications in somatic gene therapy, biotech and biomedical research. Limitations or bans on GMOs in agriculture in a large part of the world teach about such risks. Indeed, scientists should restrain [themselves] from depicting unrealistic scenarios of pervasive or far-reaching engineering of the human genome (i.e., removing risk-associated variants or augmenting some biological function) when we still lack a comprehensive understanding of many of its overall functions, short of having identified the impact of localized mutations [on] the coding or regulatory potential of a gene. On the other hand, an open debate on the pros and cons of the technology and applications, and efforts at consensus-building among scientific societies and other stakeholders on what is acceptable and what falls beyond the currently acceptable boundaries (practical as well ethical) of a scientific experiment or biomedical intervention may help build better confidence on the self-correcting quality of science and open society.

Charo: It is useful to do the math when speculating on the population genetics alterations one fears might ensue. As with the germline engineering debates in the 1990s, even if the technology were used, the number of users would likely be so small as to have little or no effect on population diversity and distribution of traits.

Weihzi Ji: Gene editing in all human cells, not only of germline cells, creates social challenges. First, if gene editing is expensive, only rich people will be able to afford it. That means these gene improvements are available only to the richest societies, and only [the] richest people are able to have 'less-sick' babies and with it enhancements become possible, 'more beautiful and intelligent' babies. Another problem is that engineering may counteract natural selection in populations and cause unanticipated effects on diversity of human variants in [the] gene pool. Third, there is no doubt that this technology will bring with it the means for prolonging life through improved medical care. How to deal with resource consumption is a huge challenge. In my opinion, the greatest potential societal benefit is to rid society of genetic diseases that create undue suffering and drain resources.

Weihzi Ji

Bredenoord: We live in a technological culture. Biomedical technology is like any other technology—it impacts society. Usually, a distinction can be made between soft impacts and hard impacts. Hard impacts typically include safety aspects, economic aspects and cost-effectiveness. Soft impacts include the impact a novel technique has on our moral actions, experiences, perceptions, interactions with others and quality of life. It is too early to discern the societal (soft and hard) risks and benefits of germline engineering. That said, I would venture the following as potential societal risks: public pressure to use this technique (which would reduce rather than enhance autonomy); how to pay for this technology; how the use of the technology for enhancement would affect society; and safety issues arising from premature clinical applications and misuse. In terms of societal benefits, I would suggest that the technology may offer curative treatments for sometimes devastating diseases and alleviate human suffering and improve the quality of life.

Zhang: It is important to thoughtfully evaluate the ethical implications of germline editing. Where do we draw the boundary of what is an acceptable biological trait for editing

in the germline and what is not? If we get to a stage where we feel that there is enough understanding of the technology, the first diseases that will be tackled will likely be the most grievous kinds (cystic fibrosis, sickle cell anemia, etc.). However, as we become more comfortable with the safety of germline editing, should we allow editing to remove mutations that do not cause early-onset disease but may in combination with other factors increase risk for late-onset diseases like Alzheimer's? What about more manageable diseases like diabetes? What about height, appearance and intelligence? Where do we draw the line? These are enormously complex questions, and we need to engage the society and a wide variety of experts to fully consider all possible issues.

Martin Pera: The risks include the unanticipated consequences of genetic intervention (variant alleles may have important advantages in some situations that we cannot anticipate). Also, in some instances—for instance, correction of hearing deficits or enhancement of stature—patient groups have argued that the 'defect' is a perfectly acceptable form of human variation that should not be subjected to genetic cleansing.

Martin Pera

In what cases would you consider human germline engineering ethically acceptable?

Naldini: Potentially and only for the *in situ* correction of a well-established genetic mutation causing with high penetrance a severe to lethal disease lacking effective treatment, and provided that editing aims to restore the common wild-type allele.

Greely: If it were proven sufficiently safe, I think the strongest case for it being ethical would be when there is no other way that a particular couple could have a healthy child that was genetically 'theirs'.

Feng: I would support germline engineering only if there is a clear case for preventing severe illnesses, and there is no way to select healthy oocytes for IVF. This could be rather rare.

Charpentier: I believe the European Convention for the Protection of Human Rights and Dignity of the Human Being, which states that "an intervention seeking to modify the human genome may only be undertaken for preventive, diagnostic or therapeutic purposes and only if its aim is not to introduce any modification in the genome of any descendants," is a potential path forward, assuming very high safety standards and no alternative treatment options being available. Having said this, personally I have concerns regarding the modification of germlines in humans.

Emmanuelle Charpentier

Lovell-Badge: Germline engineering is only ethically acceptable if it is safe. But if it is safe, then I, and perhaps society at large, would probably not object to use of the techniques to avoid a serious genetic disease and in instances where preimplantation genetic diagnosis is not appropriate, such as in the unlikely situation someone is homozygous for a lethal mutation (e.g., Huntington disease). It may even be appropriate as a means to avoid a less serious condition that will have a transgenerational effect and be a significant concern to the family (e.g., mutations of genes on the Y chromosome that reduce male fertility to such an extent that it is necessary to carry out intracytoplasmic sperm injection to have children, not just [for] the individual but all his male descendants). Correcting such a mutation to allow male children to be fertile would be ethical in my view. Enhancement is trickier. Using these methods to confer disease resistance may be considered okay: who would not want their children to be resistant to HIV, Ebola, etc.? The situation is less clear for diseases with a strong genetic risk factor. For example, the *APOE4* allele of the apolipoprotein E gene is associated with Alzheimer's disease; heterozygotes are approximately 3 times and homozygotes 15 times more likely to develop the disease, and to do so earlier, than individuals homozygous for the common *APOE3* allele, and where the *APOE2* allele may even be protective. Why not use gene editing to change *APOE4* to *APOE3* or *APOE2*? However, it's unclear how *APOE4* confers risk and furthermore, with any risk allele, particularly a common one, it is important to ask why it is maintained in the population at a relatively high frequency; could *APOE4* in fact confer some advantage to carriers unrelated to its connection to Alzheimer's? Moreover, parents are always seeking ways to give their children an advantage in life, and we do not consider this unethical. Sending a kid to a good school, for example, can have a transgenerational effect. However, a germline genetic change may be passed down without subsequent generations having a choice (except the same technology could be used to reverse the enhancement).

Bredenoord: Translating germline modification into clinical trials and society requires time, careful research (involving both the science and ethics) and public deliberation. Broadly, I would propose two conditions for an ethical use of germline engineering. First, there is a requirement for safety. First-in-man use for germline modification is ethically challenging by nature, particularly because the needed evidence to reliably predict risk and benefit (testing in humans) is missing. This needs careful, long-term, interdisciplinary research and sufficient evidence to make the leap from bench to bedside. It also needs more ethics research, particularly in determining when an acceptable risk-benefit balance has been reached. Second, one of the most prominent (nonsafety) objections against germline modification is the fear that it would become possible to alter so-called 'essential characteristics' of a future person. This could violate—what philosopher Joel Feinberg has coined in another context—the child's right to an open future. I have argued previously that a clinical application of germline modification could still be compatible with the position that one should not violate the child's right to an open future. To prevent that a child is predetermined toward a specific plan of life, it seems reasonable to only allow modification that broadens so-called 'general purpose means'. These are capacities that are useful and valuable for carrying out nearly all plans of life. In other words, we should only allow genetic modifications that we can assume give children traits that are useful for all conceptions of a good life. Although debate is possible (and necessary) about what general purpose means exactly are, being healthy should clearly be included. Health, after all, is a *sine qua non* for many (though not all) plans of life.

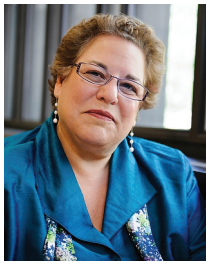
Annelien Bredenoord

What do you consider the optimal approach for oversight: full international ban, temporary moratorium, regulation or *laissez faire*?

Jacob Corn

Jacob Corn: I, and the other authors of the *Science* Perspective, am asking for a temporary moratorium on human germline editing research while a wider discussion among representative stakeholders from a variety of areas is under way. We are in the process of initiating a larger meeting for just such a purpose (see comment from Robin Lovell-Badge in **Supplementary Comments**).

Charo: As to the research on gametes or embryos, international legal harmonization is unlikely, given the varying legislative and regulatory schema. In many places, some or all of this research would be completely illegal, in others it would be regulated and in others it would be possible without any independent oversight. Even within the United States, some variations in state laws are relevant. This is why an initial step involves public discussion and development of principles to guide the research.



Alta Charo

Qi Zhou: I think a temporary moratorium is the optimal approach. We should put our current efforts into solving the technical problems and testing the safety and efficacy of germline engineering treatment with animal experiments, but we can leave the door open for germline modification for future application in curing some severe diseases.

G. Steven Martin

Doudna, Carroll, Martin & Botchan: We don't think an international ban would be effective by itself; it is likely some people would ignore it. Regulation is essential to ensure that dangerous, trivial or cosmetic uses are not pursued. In addition, a broad discussion of the prospects and limitations will have two positive effects: first, it will alert people broadly to the concerns about the current technology and potential long-term effects; and second, it will encourage people who are eager to use the technology that there is a path to applications, so they should delay its application until the concerns have been more thoroughly examined.

Edward Lanphier

Lanphier: We favor a moratorium on genome editing research on human germ cells while the pros and cons of this technology application are discussed, a determination is made as to whether or not there are any good arguments in favor of moving forward, and if so, clear guidelines are established for specific cases in which germline genome editing could be used.

Perry: In the United Kingdom, the Human Fertilization and Embryology Act covers all generation of human embryos outside the body and as such includes germline engineering procedures. Given this, no new legislation is required in the UK to regulate human germline engineering unless it becomes possible to engineer genomes *in vivo*. It seems unlikely that a full international ban would ever be agreed [to] and even if it were, it's unclear to me how it would be policed. This debate cannot be seen in isolation: for example, China would be less inclined to listen to the United States regarding human germline engineering if political relations were otherwise deteriorating. Arguably, the emphasis should be on discussion, not a moratorium. If the prevailing view to emerge following discussion is that there should be a moratorium, so be it. However, a moratorium may drive research underground when what is needed is the opposite: open and transparent communication of a measured international research effort. Champions of a temporary moratorium should make it quite clear as to the circumstances under which it would be lifted. A moratorium may evolve into prohibition and 'illegalization'; it could stifle debate and have unintended consequences, including 'genome engineering tourism' to lax sovereignties, leading to untested and poorly regulated procedures. There may be some parallels with discussions about legislation for abortion and euthanasia in this regard.

Pera: I think a moratorium to enable a full and reasoned debate, and to allow for education of the public, is essential. It is too early for regulation, including an international ban, and *laissez faire* is too risky. With reproductive cloning, scientists agreed to a ban, but reproductive cloning was different in that it was very difficult to envision any good medical rationale for undertaking it.

Is it possible to have an Asilomar-type resolution today, given the questions swirling around CRISPR germline engineering, the international nature of research, and ease of use of technology and rise of 'garage' biology outside of traditional centers?

Moreno: There's a nearly reflexive tendency to think of Asilomar, but Asilomar has become for biology what Woodstock has become for youth culture—a mythology that's grown but that obscures how muddy the event itself was at the time.

Kim: I am skeptical about an Asilomar-type resolution. Several decades ago, recombinant DNA technology was available to a limited number of labs in the United States. Now CRISPR genome editing is used widely all over the world. CRISPR has democratized genome editing. Human germline genome editing cannot be performed in a garage because it is illegal to obtain and manipulate human eggs in most developed countries.

Jin-Soo Kim

Zhou: I think an Asilomar-type conference involving scientists in different countries is a useful way to draw some consensually agreed guidelines to address this question.

Bosley: While the world has changed a lot since 1975, I think that leadership still matters. In fact, given the international nature of research and the ease of [using the] technology, it may matter even more than it did in 1975. I think there's an interesting question of how to engage across all of these diverse parts of the scientific community, and that is the challenge of how to effectively lead today. Leaders engaging on this topic are already emerging from long-established and highly respected academic institutions—that's not surprising; that kind of leadership is in their DNA and they're really good at it. But

how can the 'garage' biologists, for example, also be part of the leadership on this question? I think genuine and broad engagement will be key. Whether it's an Asilomar-type resolution or another forum or tool—or indeed, many different forums and tools—leadership and an ongoing dialog do matter. This isn't the kind of question that can be addressed with one resolution or one conversation, and people's perspectives may well evolve over time.

Feng: I think it is possible and important, even if we cannot get every country together. It is very important to have this meeting early (right now) and have some countries lead the way. Including both developed and developing countries in the leading group will be critical.

Perry: An Asilomar-type meeting seems unlikely. One has to compare the circumstances surrounding Asilomar and the human germline debate. In 1975, Asia was not such an economic and scientific powerhouse. The language describing recombinant plasmids and viruses resonated with the fear of a cancer-causing infectious outbreak. This is not directly relevant to human germline engineering, but it is instructive. Asilomar reflected a deep concern that recombinant DNA had terrible potential, so parallels with Asilomar may reveal an unstated premise of the proposed moratorium for human germline genome modification—that it is, in essence, bad. But the premise seems to ignore the potential for good of human germline genome modification. Was there an analogous awareness in the debate of 1975 that good could come from molecular cloning? Every day that a moratorium delays development of human germline genome modification is potentially a day it adds to human misery. Two general points are also related to this question. First, the United States does not hold the same sway as it did in 1975. The second point comes by way of precedent. Given the considerable lag between the false claim of Hwang Woo-suk to have generated human nuclear-transfer embryonic stem cells and the first verified report almost a decade later—and notwithstanding the assortment of attention seekers, kooks and loons who have claimed to be performing human cloning in the past 15 years but then turned out to be nothing more than an assortment of attention-seekers, kooks and loons all along—the 'garage biology' idea may be less likely than it's given credit for. This is not an argument for complacency, but for a realistic take on what is likely.

Rosario Isasi & Bartha Knoppers: Perhaps it would be reasonable to adopt a tiered approach, encompassing a temporary ban on any research and clinical activity directed at intentional human inheritable genome modification, while at the same time allowing nongermline modifications. Or conceivably, is a more plausible approach a temporary (or permanent?) prohibition on initiating a pregnancy with a human embryo whose germline has been altered? An expedient, albeit knee-jerk, approach would be simply legally prohibiting intentional germline and non-germline genome modification based on fears over slippery slopes resulting in eugenic scenarios.

Left: Rosario Isasi. Right: Bartha Knoppers

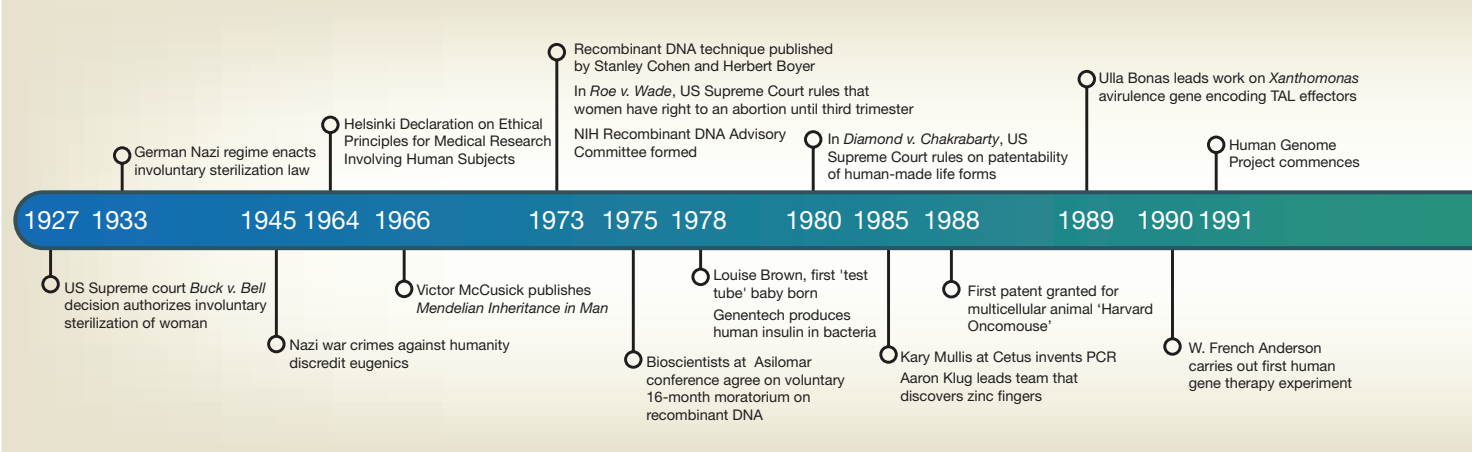
Venter: An Asilomar type conference or the equivalent will make some feel better while extending the illusion that they can influence the applications of a simply applied technology to a key human need. Only by greatly increasing our understanding of the human genome and genotype-phenotype relationships and the consequences of making changes will we have the knowledge to make wise decisions. Until that time, human genome editing should be considered random human experimentation. We should push off the inevitable as long as possible to gain time to gather the knowledge and wisdom to enable us to proceed to the benefit of our species.

Does the fact that CRISPR technology works relatively easily in different laboratories across the world have an impact on the effectiveness of a ban or moratorium and pose different issues than germline gene therapy or reproductive cloning?

Hank Greely

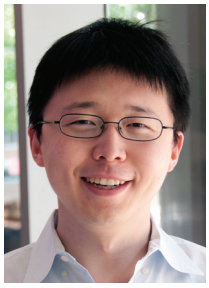
Greely: Of course, the ease with which the technology can be used makes it harder to impose a ban. CRISPR or any present or future equivalents would be a way of doing germline gene therapy that holds out the possibility of doing something that is much more effective than current gene therapy methods or than reproductive cloning.

Corn: I think responsible scientists will respect significant, widespread concerns about germline editing. It remains to be seen how the ease of use of CRISPR will impact clinical



use. While garage applications are not realistic, one could imagine a future in which most well-equipped medical centers might have access [to] things like somatic (e.g., hematopoietic) cell editing.

Lanphier: Yes, the fact that there is an easy to use system for genome editing, such as CRISPR, creates a low barrier to entry for germline genome editing and means that a ban or moratorium may not be easily enforced and thus not completely effective worldwide. While the CRISPR-Cas9 system has not been shown to be reliably specific, it offers a more straightforward approach for targeted manipulation of the genome than germline gene therapy or reproductive cloning.



Feng Zhang

Zhang: It is important to educate the scientific community and the public with regard to the implications of genome editing. This way people will be best equipped to make the most ethical and sensible decisions in their own research as well as monitor activities around them. Technically, CRISPR is not simpler than germline gene therapy or reproductive cloning, and it is not more or less challenging to regulate.

Perry: The ease with which the Cas9 technology can be used, coupled with its clear potential may make any moratorium less effective; whatever is being said publicly, there may be a behind-the-scenes race to develop the technology to gain an advantage before the moratorium is lifted. I see this as likely and unpoliceable. On one hand, this may be precisely what some people wish. On the other, the result may be diametrically opposite to what others wish. An alternative would be to pursue the work and in parallel foster an environment of openness, transparency and trust. As to 'garage' biology, reproductive cloning may be instructive: we're still in the tall grass getting on for 20 years after the first authenticated mammalian cloning was reported and few people can do it in any species.

Pera: No, because genetic manipulation is only part of the story. It will still be necessary to carry out medical procedures to successfully deliver modified gametes or embryos into the human reproductive cycle, and this cannot be done in isolation by one or two individuals.

The UK has approved mitochondrial replacement therapy and there was a recent report of human somatic cell nuclear transfer into an enucleated oocyte; how different compared to these are the ethical challenges posed by CRISPR germline engineering?

Ji: CRISPR germline engineering has additional ethical challenges to mitochondrial replacement. One of these is if we should change our genome before we really know all the functions of our genes and of our genome; of course, 'junk DNA' is not entirely junk.

Feng: The major difference is that in the UK case, one does not change the gene pool. It changes the genome of a human, but not the human race.

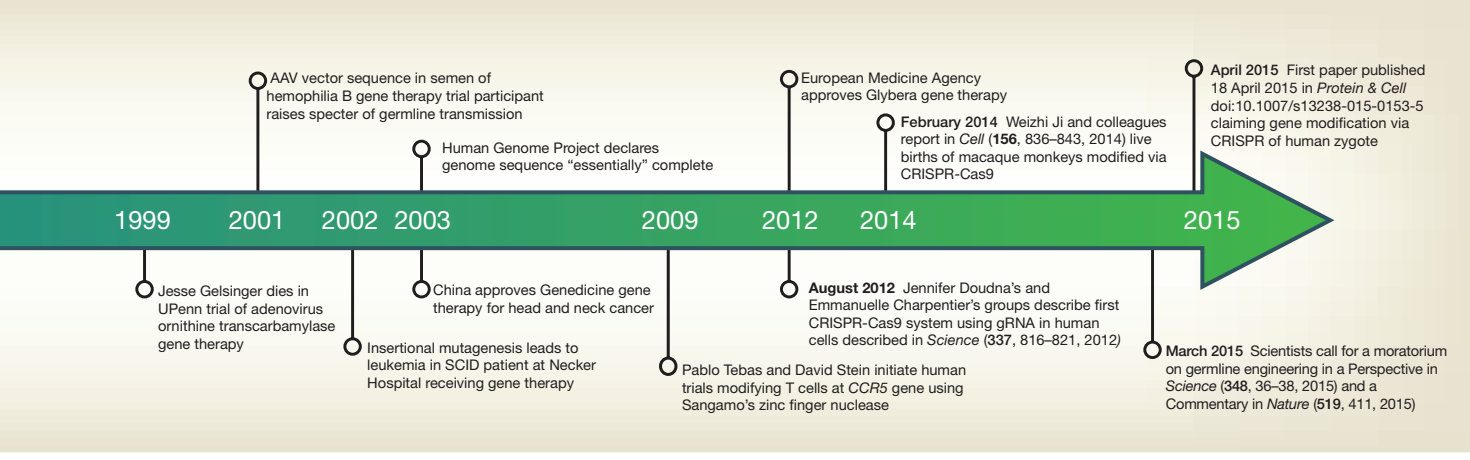
Zhou: Germline engineering via CRISPRs or other genome-editing technology faces bigger challenges than mitochondrial replacement therapy because mitochondrial DNA carries much less genetic information than genomic DNA. The ethical challenges are the same, however. Do we allow such biomedical approaches to be used to achieve genetic enhancement of future generations? Human therapeutic cloning does not directly involve germline changes. For human reproductive cloning, I think the scientific community and governments all over the world have already reached a consensus that it should be banned completely.

Lovell-Badge: The ethical and technical challenges are different and should be treated as such.

Doudna, Carroll, Martin & Botchan: Because mitochondrial transfer is permanent, there will be unpredicted effects of novel alleles in a given background, similar to novel alleles generated by CRISPR engineering of the germline. However, there are significant differences between the two approaches: first, there are very few genes in mitochondria, and they have well-defined roles specific to that organelle, so there are fewer places to go wrong. Second, no nuclease-based engineering is involved, so there will be no off-target mutagenesis. Finally, unlike the nuclear genome, deleterious effects in transplanted mitochondria cannot be moderated by sexual reproduction because the organelle is inherited uniparentally.

Mike Botchan

Bosley: The United Kingdom's recent action was the culmination of deep debate and extensive consideration over a long period of time. It's a good example of engaging diverse constituencies and considering the implications from many different angles. These techniques do involve germline changes, but for several technical reasons, their implications are much more



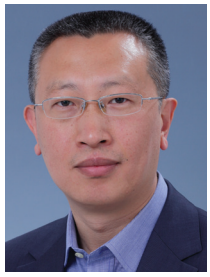
constrained than the CRISPR-Cas9 technology. With mitochondrial replacement, only a very limited number of genes are involved, the technique is such that it can't extend to more genes than the mitochondrial ones and the diseases caused by mutations in those genes are very severe. The balance of potential benefit to patients and broader implications is one that can be assessed, understood and a judgment made about whether that balance is acceptable. And the UK government made that judgment with their approval of it. The current question about CRISPR and germline engineering is far more complex, and we don't have a sense of the breadth of the implications, and we don't understand the risks well. The technology's progress now demands us to confront these questions, but that can't be done quickly.

Perry: Mitochondrial replacement and nuclear transfer are different from Cas9-mediated germline engineering and seem to be red herrings in the debate. Indeed, there is a danger that discussion of germline engineering will be addled by them. Why? First, because mitochondrial replacement doesn't alter DNA sequences, it mixes up mitochondrial and nuclear genomes in a new combination that arguably could have occurred naturally. Also it's not new. Others have been doing this kind of thing for ~15 years or more. It's possible—likely, even—that had the timing of the UK legislation not coincided with recent advances in Cas9, we wouldn't be thinking about it. Somatic cell nuclear transfer also doesn't change genomic sequence; on the contrary, it preserves a preexisting nuclear genome produced naturally by meiosis. I don't think advocates of therapeutic cloning have put 'generating germ cells for genetic alteration' at the top of their list of justifications, but otherwise nuclear transfer ES cells are also of limited relevance to discussions about human germline genome engineering.

Is international, national oversight or a combination of both needed, and which do you consider the correct regulatory or government agencies to oversee this research?

Moreno: There's a great deal of regulatory diversity under which gene editing could be brought among the countries that have the best developed science capacity (e.g., on embryo research, GMO, etc. and if these techniques are as easily accessible as they seem to be it won't be hard to go 'offshore'). Unfortunately, the international regimes for life sciences regulation are few to none, once one gets beyond intellectual property and some research ethics standards, especially as concerns sanctions for bad behavior. Witness the wholly voluntary nature of the handling of the ongoing controversy about gain-of-function research. Again as to sanctions, research funding can be withdrawn but it looks like systems like CRISPR can be done for rather little money. For demonstrable harms after the fact, there is little redress; the United States is not a part of the International Criminal Court, for example. Nonetheless, there should be some global forum for the exchange of views about germline engineering. A natural venue would be UNESCO's International Bioethics Commission (of which I happen to be the US member), especially in light of Article 16 of the Universal Declaration on Bioethics and Human Rights (2003): "Article 16—Protecting future generations: the impact of life sciences on future generations, including on their genetic constitution, should be given due regard." The result of such an exchange could be a new declaration or perhaps an addendum that takes gene editing into account, one that would bind the states' parties.

Naldini: Oversight by legitimate ruling bodies representing all society's stakeholders should suffice upon informed advice by scientific societies or representatives. Scientific societies and communities should hold a debate and express general recommendations.



Qi Zhou

Zhou: I would prefer 'international guidelines plus national oversight policies' to oversee human germline engineering. Medical or healthcare services, academic institutions and industry face the same scientific and technical barriers, but the ethical challenges are different in different countries due to differences in society, religion, economics, etc. Thus, international guidelines could make a guide for the consensus questions and provide a basis for each country to formulate its own oversight policies, according to its own realities and cultural, political, religious and social context.

Robin Lovell-Badge

Lovell-Badge: National oversight should suffice, except many countries do not have a system in place to do this. I very much doubt that international bodies would be either reasonable or effective unless they work by consensus, are driven by science and [are] listened to by clinicians.

Charpentier: Living in a globalized world as we do these days, any isolated national initiative might fall short over time.

Dana Carroll

Doudna, Carroll, Martin & Botchan: In the United States, the Recombinant DNA Advisory Committee now reviews all proposals for gene therapy, including ones using designer nucleases (no CRISPR protocols have been submitted, as far as we know). The FDA also reviews such proposals because genes and nucleases are viewed as drugs. It would be good to have agreed-upon standards internationally.

Perry: It's a matter of trust, and it's not clear to me whether the foundations for such trust exist. The UK and possibly other countries may benefit from a 'go-to' source of disinterested and reliable information, for example, communicating advances in the genome engineering toolkit, identifying benefits to humans and animals (veterinary medicine), defining fully and partially prescriptive genome editing and explaining the law. It would seek [to] neutralize disinformation and help manage public expectations regarding safety, indicate realistic time-frames and explain the need for animal experimentation. It might address minimum standards to prevent corner-cutting experimentally or in clinical trials, how nonediting technologies (especially whole-genome sequencing) will be reckoned and whether there is a meaningful distinction between, say, single-gene heritable disease 'correction' and IQ 'correction'. If this could be done internationally, all the better.

IVF, *in vitro* fertilization; ES, embryonic stem; GMOs, genetically modified organisms; NHEJ, nonhomologous end joining; HDR, homology-directed repair; ZFNs, zinc finger nucleases; TALENs, transcription activator–like effector nucleases; ICSI, intracytoplasmic sperm injection UNESCO, United Nations Educational, Scientific and Cultural Organization; FDA, US Food and Drug Administration.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper (doi:10.1038/nbt.3227).