With rumors circulating of CRISPR-Cas9 mediated human germline engineering, are we at a new "Asilomar Moment"? In a letter to *Science* last month "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 explores the issues with a Q&A:

1. ***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 germ-line engineering is inevitable?***

Jonathan Morena: I think it is inevitable, not only for medical research but also -- and perhaps much sooner -- for agriculture. These techniques appear to be far more efficient for breeding desirable animals than SCNT and also for livestock protection.

Hank Greely: It's likely to be inevitable, though perhaps only in very limited cases.

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.

Jacob Corn: When it comes to germ-line engineering, we are masters of our own destiny. The sun rising and setting every day is inevitable. Germ-line engineering is a choice we have the opportunity to make. The purpose of our Perspective in *Science* was to encourage researchers to slow down, ask difficult questions beyond the science, and make a conscious and well-considered decision on this front.

Jinsong Li: In my opinion, the idea of human germ-line-mediated gene engineering will come true in future. To date, there are two strategies, which have been successfully demonstrated in mouse for use of CIRPSR-Cas9 system to correct genetic diseases in germ cells, which could probably be used in human in future. The first strategy, in which, CRISPR-Cas9 has been directly transfected into zygotes (Wu et al, *Cell Stem Cell*, 2013, 13, 659), has two barriers that could not be easily overcome, leading this strategy unacceptable to be considered curing a human disease. First, as you can see from our paper or other published papers on generation of gene-modified animals via direct injection of CRISPR-Cas9 into zygotes, not all resulting pups carry expected genotype, which is not acceptable for human genetic disease rescue. Second, off-target effects, although very rare in our study and others, still exist in the resulting pups, which is also not acceptable for therapeutic application. To circumvent these two shortcomings, in the second strategy, the disease-causing genetic defects could be repaired in germ stem cells, which may produce gametes carrying corrected genes, thus transmitting to progeny. This strategy may make it possible to transfer therapeutic application of CRISPR-Cas9 in curing genetic diseases from mice to human. We have demonstrated that efficient gene editing in mouse spermatogenesis stem cells (SSCs) by CRISPR-Cas9 system, and provided the proof of principle of curing a genetic disease via gene correction in SSCs (Wu et al, 2015, *Cell Research*, 25, 67). Taken together, we believe that germ stem/progenitor cell-mediated gene engineering might be

Nature Biotechnology: doi:10.1038/nbt.3227

an appropriate strategy for treating human genetic diseases. While we support the application of gene-modification techniques in human genetic disease treatment through germ cells, we do not support their applications for non-medical purposes.

Emmanuelle Charpentier: I believe that for the time being germ-line engineering is evitable. There are simply too many unanswered questions, which still need addressing. Most European countries have ratified a convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine that strictly forbids the manipulation of the genome of human germ cells when those manipulations are within the frame of medically assisted procreation. "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."

Weizhi Ji: Three years after its initial development, CRISPR technology is widely used by biologists, and already many offspring have been born from gene edited animals, including monkeys. Only once scientists and medical practitioners have understood and considered all of the biological and ethical consequences of CRISPR-based gene editing for genetic disease in the germline of humans should gene editing in the human germline be considered. Before applying this technology to human beings, especially the human germline, a great deal of work should be done to evaluate the safety of this technology. I think the most optimal model is monkey.

Jin-Soo Kim: I believe that human germline engineering will be practiced sometime in the not-too-distant future to prevent transmission of a fatal mutation to newborns.

Qi Zhou: Though the germline engineering technologies are developing very fast, many problems remain unresolved regarding the technology, ethics, public policies and social issues. So I think it is still too early to apply the techniques to engineer human germline cells, even for pure therapeutic applications. Until the safety and efficacy questions are thoroughly examined in animal experiments, and the international community has agreed on rules for regulatory oversight, human germline engineering should be prohibited.

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.

Jennifer Doudna, Dana Carroll, G. Steven Martin, Mike Botchan: It seems inevitable because the basic technology is essentially all in place. That was a large part of the motivation for the Napa meeting and the *Science* Perspective.

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 germ-line modification would not involve gene editing techniques by mitochondrial gene transfer (also referred to as mitochondrial donation or mitochondrial gene therapy). Recently, the UK Parliament legalized this technique aimed at preventing the transmission of mitochondrial DNA mutations from mother to child. The US FDA has requested the Institute of Medicine to produce a consensus

report regarding the ethical and policy issues of mitochondrial gene transfer (I attended the first workshop last week in Washington DC).

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 have been developed over many years and are part of what scientists learn as part of their training.

Research scientists and clinicians take these and many other factors into account in the work they do every day, and I think that will be the case here as well, particularly given that the considerations surrounding human germ-line engineering are broad and profound.

Human germ-line 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 dialogue 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 dialogue 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.

The power and potential of this technology is amazing. It seems that almost every day a new idea emerges about how to use it. I think we have a responsibility both to find the right way to realize that potential and also to do it in a way that is highly ethical.

Feng Zhang: There are a lot of advances being made in genome editing, IVF, and germ stem cell research. In principle, being able to remove a known disease-causing mutation from the genome of germ cells might be a way to prevent grievous illnesses, however, it is not nearly that simple. First, genetic diagnosis and screening through IVF provides an effective solution for the majority of cases. Second, given that many diseases might be treatable through somatic cell genome editing, it is unclear whether germ line editing is an appropriate solution. Third, from any perspective, technical, scientific, or ethical, we are still far away from being able to carry out germline genome editing-based therapies. Nevertheless, it is important to begin discussions and grapple with the multitude of challenging questions now.

***G*uoping Feng** I think this for sure will happen, for correcting defective genes.

Tony Perry: Human germline genome engineering is probably inevitable although it's unclear how quickly it will come about. There are three issues: the tools, the goals and whether the tools can achieve the goals. We should soon have the tools. The goals are a major focus of the ethical debate that will determine when/if human germline genome engineering is implemented. There

Nature Biotechnology: doi:10.1038/nbt.3227

may be insuperable barriers to the tools achieving complex goals like higher IQ compared with, say, the modification of a single nucleotide to prevent a disease.

Martin Pera: I think we will certainly develop the capability to carry out germ line engineering in the human. However, the ultimate application of this technology will require a good deal of careful thought and debate. It is best if the public discussion begins now, so that a reasoned approach, rather than one based on an immediate reaction to sensationalistic headlines, will chart the way forward.

Edward Lanphier: Human germ-line engineering is inevitable. Other technology (ZFNs) has been available for years and has been used successfully to modify the germlines of animals such as rodents and pigs. However, the recent advent of CRISPR/Cas9 technology makes the possibility of germline genome editing much more accessible to a wider range of individuals.

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 germ-line 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. Currently preimplantation selection is effective in choosing cells with desired traits. The issues will be associated with the efficiency of the editing technology and percentage of live births with the desired traits vs unintended consequences. However, It will be complicated to establish if genome editing techniques result in negative side effects against a widely varied genetic background. Public perception of increased abilities or the absence of disease will create an unlimited demand for genome editing services globally. One only needs to look at the proliferation of stem cell therapy clinics around the world largely in the absence of clear cut clinical data.

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

Moreno: I will defer to the real geneticists here but getting the targeting right seems to be a problem, as it has been in previous gene-therapy experiments like the ones for SCID.

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 germ-line 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/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/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 towards achieving efficient editing in primary cell types are efficient delivery of the gene targeting machinery, tolerance and permissiveness/proficiency of the treated cell to homologous recombination, selection of the desired edit, possibly epigenetic scar at the targeted gene altering expression features.

Greely: Proof that the process is safe. That will mainly be limiting off target effects but there would also have to be a showing that it was safe against other possibly unforeseen effects of such an intervention in a gamete or a zygote. I'd expect a great deal of preclinical work, in human materials in vitro as well as in non-human animals, including certainly primate and, for this intervention, perhaps even non-human apes.

Corn: My hunch is that the bottlenecks have moved. Genome editing was once a major barrier, and that's no longer the case. Now I would speculate that downstream safety and efficacy matters are more limiting than making the edit itself.

Jinsong Li: There are at least three outstanding technical barriers that need to be solved before the application of SSC-mediated gene therapy in human. First, how to achieve efficient derivation of SSC lines in humans remains a major problem. Second, whether it will be possible to obtain mature sperm from cultured SSCs remains uncertain. And third, whether it will be possible to achieve efficient genetic modifications in SSCs in humans also remains unclear. In my mind, there is still a long-way to go to use CRISPR-Cas9 to correct genetic diseases in human via germ cells.

Emmanuelle Charpentier: Besides a very significant number of ethical questions to be addressed, safety concerns are probably the most pressing consideration. I assume that in the future gene editing may be sufficiently specific to its target sequence(s); we are currently not in the position to categorically exclude off-target activities.

Weizhi Ji: I think most of the technical barriers are related to off-target. Although many strategies have been developed to enhance precise genome engineering, we still have a long way to go.

Cohen With CRISPR, there are few technical barriers to altering germlines. The barriers have more to do with unintended, not yet understood consequences of altering, deleting or inserting

particular genes, or combinations of genes, and the lack of understanding of the impact of more than a single alteration.

Kim: Before moving to germline editing, researchers need to develop, first, methods to suppress error-prone non-homologous end joining (NHEJ) and to enhance the efficiency of homology-directed recombination (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/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%.

Qi Zhou: I think there are two major technical barriers. One is how to increase the precision and efficiency of genome editing technology so that one avoids off-target modifications and genetic mosaicism. The other major concern is the potential unanticipated consequences of the on-target modifications, due to the complexity of gene regulatory networks and our limited knowledge on the mechanisms of many genetic traits and diseases.

Lovell-Badge: First, assessing the fidelity of the genetic change being made at the locus being targeted with gene editing techniques, which is not always as desired. Second, our inability to know the level of off-target effects without carrying out experiments in the human germline, which might be specific to each gene editing construct. For example, if the method is zygote injection of CRISPR, then it would be necessary to derive ES cells from resulting early embryos and carry out genome sequencing. Even then, it is not going to be feasible to test for off-target effects in the context of the genome to be targeted if this is always going to be a new zygote. Third, how to avoid mosaicism if the gene editing only occurs in some cells. Fourth, how to move from using gene editing methods to knockout genes (relatively easy) to correcting or altering genes (which is much harder and much less efficient). Although simple mutations can be of clinical benefit in some cases, I would imagine that most desired clinical applications would be to correct mutations or change a risk allele to one that is protective.

Doudna, Carroll, Martin, Botchan

1. Many of the clinical applications will require repair of the Cas9-induced break by homologous recombination; at present this is quite inefficient in many cell types and may be so in human embryos.
2. We don't currently have full control over precisely what happens during repair at the intended target.
3. There are significant concerns about unintended cleavage at secondary targets, and we cannot confidently identify all of those potential off-target sites.

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 are. Do they 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

Nature Biotechnology: doi:10.1038/nbt.3227

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.

Feng: One of the major issues is the off-target effect. A second issue is the potential mosaicism from editing after single cell stage. Another issue is the low efficiency of homologous-directed repair (HDR) for correcting genetic defects. However, these are technical barriers that will be solved in the near future. In fact, progresses have been made in each of the areas, such as using double nickases to reduce off-target effects, using Cas9 or nickase protein instead of mRNA for faster action, and suppressing genetic programs to increase HDR efficiency.

Tony Perry: Fidelity: generating a system specific at an acceptable level to the intended target. Also, in the initial stages of applying the technology, at least, confirmation: using single blastomere diagnostic sequencing safely and accurately to verify that no non-prescribed changes have been made.

Pera: The barriers depend on the technology. If we consider germ line modification in eggs or early embryo cells, which is already being undertaken in animals, then the main considerations are the precision of the targeting approach and off target effects. An even more complex issue, which has not been widely considered is how to monitor off target effects and gauge their potential biological impact. If we think of using stem cell technology to produce gametes, then we will have to learn how to produce functional normal gametes, in addition to overcoming the issues around precise targeting.

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.

3. What are the individual health risks associated with germline engineering (e.g off target effects, genetic chimerism, unanticipated effects of on-target changes) and what are the potential individual benefits?

Naldini: Risks are mostly associated to off target activity of the nucleases, which remains to be investigated thoroughly, especially for the more recently developed RNA-based platforms, although it is likely that highly specific reagents will soon be available and alleviate the risk (as it has happened with the earlier protein-based platforms, such as ZFNs and TALENs). Chimerism remains a major issue, as discussed above.

The benefits are not obvious as compared to currently available embryo screening methods to address the risk of transmitting a disease-causing gene from identified carriers. Purging/editing a risk-associated allelic variant seems unlikely to reach an acceptable risk/benefit ratio. Augmentation (if feasible) falls beyond the acceptable scope of most biomedical research or medical intervention.

Nature Biotechnology: doi:10.1038/nbt.3227

Greely: I think you've listed the anticipated individual health risks; I'd add "unanticipated." It may be that the process of this intervention, in gametes, gamete precursors, zygotes, etc. would 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. It would be if a person who is homozygous for dominant diseases or a couple, where both have the same autosomal recessive disease, want to have genetic children. There may be a few other situations, but not many. The advantage that your descendants wouldn't have to use PGD seems pretty small to me. In terms of enhancement, we're so far from knowing and understanding "enhancing" genes that at this point the individual benefits are asymptotic to zero.

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

Corn: To a large extent this depends on the biology at hand. Just as there is no one way for an IND to proceed to becoming a marketed drug, there is no one path forward for either somatic or germline genome editing. The potential benefits are enormous. We are talking about cures for diseases, in which the cure itself is passed down through generations. Sobering stuff.

Li: In terms of generating gene-modified animals via direct injection of CRISPR-Cas9 into zygotes, the risks include a failure of all resulting pups to carry the expected genotype, which is not acceptable for human genetic disease rescue. Second, off-target effects, although very rare in our study and others, still exist in the resulting pups.

Charpentier: I already referred to off-target effects under bullet point 2. You are likely aware of the debate and lastly approval of the legislation of mitochondrial replacement approaches for IVF in the UK. Even under those circumstances where 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 off-spring was dismissed for humans. Potential benefits are related to the gene correction of severe genetic disease allowing kids a normal life.

Kim: Most genetic diseases caused by a monogenic recessive mutation can be cured by genome editing in just a subset of cells. Mosaicism, often observed in animals, will rarely be a problem in germline therapy. Genetic diseases caused by a dominant mutation will be much more challenging to address because it would be very difficult to correct all the cells in newborn babies. Off-target mutations would not be a huge concern when one uses paired Cas9 nickases, which rarely induce off-target mutations in a clone.

Zhou: The individual health risks reside in the technical limitations. These include the potential disorders caused by off-target effects of the techniques, or even on-target effects, due to perturbation of intrinsic gene regulatory networks. At present our understanding of the genetic basis and regulatory networks of phenotypic traits and diseases is very limited. For example, a gene currently known to cause metabolic disease may someday be found to affect intelligence.

Moreover, unlike somatic genome engineering, which only alters the genomes of certain types of cells, germline genetic modification effects the whole body, which is again more likely to have unanticipated consequences. So I think we need to take full advantage of animal studies, especially non-human primate models, to determine the risks before any clinical applications are considered.

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?

Doudna, Carroll, Martin, Botchan:

1. Some applications would be confounded by on-target mutagenesis by NHEJ. For example, one could unintentionally convert sickle cell disease into beta thalassemia.
2. 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 2 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.
3. If the "edited" individual is chimeric for the intended correction, they may still have diseased cells in critical tissues.
4. 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, since it seems very unlikely to have significant consequences
5. 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.

Zhang: Risks are off-target changes in the genome; does editing affect the cell in other undesirable ways such as altering the epigenetic state of the genome and lead to other long-lasting consequences; do on-target changes have unanticipated deleterious effects biological function? With the exception of a small number of mutations that are known to cause diseases,

Nature Biotechnology: doi:10.1038/nbt.3227

we are unable to predict the biological consequence of any specific genetic change in a cell or organism. The potential benefit is the ability to completely cure grievous diseases.

Perry: The risks depend on the targeted sequence; some may enable extremely high specificity whereas others don't. Some may have serious off-target consequences if they do occur, whereas others will 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 risk of malaria. Benefits in general include eliminating many of the 3,000 or so single-gene heritable disease traits, but medics can speak to this more knowledgably. 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.

Pera: Off target effects and the unanticipated effects of targeted genetic modification are a major source of concern. We still have a great deal to learn about gene regulation and networks. No one would have worried about off target effects in non-coding RNAs a few years ago. Using this technology to correct genetic lesions with known adverse effects would seem to be more straightforward. Indeed discussion of this topic has precedent in the consideration of the application of pre-implantation genetic diagnosis of disease in assisted reproduction.

Lanphier: The list that you have provided covers most of the health risks which may also be exacerbated in future generations. It is difficult to think of a therapeutic application that either cannot currently be addressed by available techniques, such as carrier or pre-implantation screening, or for which the perceived overall benefits outweigh the potential risks.

4. What are the societal risks of germline engineering (e.g., unanticipated effects on diversity of human variants in gene pool) and what are the potential benefits?

Moreno: Perhaps the obvious health benefits for future persons are evident, as well as possible savings for health care systems for chronic conditions and disabling conditions (though presumably everyone will always die of something so those savings might be short-term). On the other hand, population biologists suggested forty 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 conditions like sickle cell and Tay-Sachs, that idea seems to have renewed resonance now. Besides the prospect of "consumer eugenics" -- driven by parental choices rather than by state order, but having similar results such as a multi-tiererd social system based on certain enhancements -- (and here I delve into the truly far out), 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 arrays of unanticipated effects. Even if these are unrealistic scenarios, they may generate fear, distrust on scientists and over-caution on the use of the current technologies, which may inhibit their full exploitation for less problematic and more fruitful applications in somatic gene therapy, biotechnology and biomedical research. Limitations/bans of GMOs in agriculture in a large part of the world teach about such risks. Indeed, scientists should restrain 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 in the coding/regulatory potential of a gene.

On the other hand, an open debate on the pros and cons of the technology/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 building better confidence on the self-correcting quality of science and open society.

Greely: Possible loss of diversity is a theoretical one, though probably not a very serious one mainly because I doubt a truly large chunk of humanity would ever have its germ line changed. Plus, if the techniques work well and we discovered that an eliminated allele were beneficial, it could probably be added back through somatic cell therapy. The "superman" and "genetic caste" fears aren't realistic given our current state of knowledge of "enhancing" alleles and may never be.

Cohen: Less risk if such engineering is confined to specific disease targets. Greater risk if allowed to progress to discretionary, “designer gene”programs. The science fiction nightmare of an Orwellian totalitarian state or an Hitlerian society employing genetic engineering to achieve only “desirable” traits could ultimately happen, though we are nowhere close to this capability today.

Corn: It's hard to imagine editing becoming so pervasive that genetic diversity would be affected in the short term (decades), but what will happen in the long term (centuries+)? These are not things we should be hand-waving about, but should be driven by data and analysis!

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.

Li I don’t see any societal risks of germline engineering if we just use it for medical purposes. One of the potential societal benefits is that the genetic defects can be completely removed from the population.

Ji: Of course, gene editing in humans cells, not only germline engineering, will create 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 richest people are able to have 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 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.

Kim: In an ideal world, germline genome editing would be available and affordable to everyone. No parents with a fatal genetic mutation would transfer their faulty gene to their children. In an unequal society, however, germline genome editing will be affordable to the rich only, resulting in a "gene divide", as predicted in the film, "GATTACA".

Lovell-Badge: Societal risks of altering the gene pool, etc., are rather a long way off. Perhaps the biggest issue will be one of social justice. Applying the methods will be costly (at least to begin with), so will only the rich benefit ? I imagine some will worry more about the use of the technologies for enhancement and the creation of an elite than they will about the use of the methods to cure or avoid disease – even though enhancement in any meaningful way is still far away (we simply do not understand enough about traits such as height, let alone intelligence). Societal benefits could include lowering the burden, financial and emotional, of reducing genetic disease, increasing disease resistance, etc.

Doudna, Carroll, Martin, Botchan:

1. There are lots genetic disease alleles that we would not miss, but adaptation depends on the existence of adequate genetic diversity. If, in the extreme, all humans had exactly the same genome sequence and if random mutations were not allowed to accumulate, the species would not be able to adapt to changes in the environment, and we don't mean specifically climate change.
2. Genetic diversity contributes largely to individual and cultural diversity, which enrich our lives.
3. We don't know enough about the role of allelic variation in most genes to be certain that a change intended to influence one trait won't also affect others. And see response #4 to question 3. Would a particular allele have the same effect in a largely Caucasian genome as in a largely African one, despite the very high level of identity among all human genomes?
4. The availability of such technology might be limited to certain populations due to financial resources and medical infrastructure, which could exacerbate cultural inequalities.

Bredenoord: We live in a "technological culture", and this is true for biomedicine as well. New biomedical technologies always impact 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 germ-line engineering, this certainly needs careful thinking through of the issues.
With the above caveat, I would suggest as potential societal risks:

Public pressure to use this technique, which would reduce rather than enhance autonomy

Reimbursement issues
Use for enhancement
Safety issues due to premature clinical applications and misuse.

Potential societal benefits:

Offering curative treatments for sometimes devastating disease

- Alleviating human suffering and improving quality of life

Feng 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 are 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 directly cause disease but serve to increase disease risk for grievous diseases like Alzheimer's disease? 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.

Feng: A major society risk is shifting from treating diseases to "improve human biology, e.g. longevity, intelligence, physical strength". Since this is germline modification, it could have disastrous long-term effects on future generations. One cannot predict what the consequences will be when modified "better" genome is combined with other millions natural variants. On the other hand, if it is simply restoring a defective gene, there are no new variants added to the gene pool.

Perry: A major societal risk is that the debate and/or legislative apparatus are hijacked by vested interests that don't represent those of society at large. These may include commercial, religious and even scientific lobbies. By way of precedent, one might argue that the GMO debate (in agriculture) has been incredibly badly handled, but there is a risk that we haven't learned from this and will make the same mistakes, potentially delaying or foreclosing on what could be an immensely powerful means to prevent human suffering.

Regarding the gene pool, this depends on the nature of the genome engineering. Can gene sequences that predispose to disease in general reasonably be said to enrich the gene pool? If not, would it matter that it's gone when in all other regards the pool is preserved? The possibility of genomic changes not directly related to disease prevention could potentially affect the gene pool but it's hard to say how and needs modeling with careful attention to ensure that input parameters are realistic.

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 instances 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.

Lanphier: Both therapeutic and "elective modifications" could, indeed, result in unanticipated effects on diversity of human variants in the gene pool. It is difficult to think of a therapeutic

application that would provide significant societal benefit that either cannot currently be addressed by available techniques, such as carrier or pre-implantation screening, or for which the perceived overall benefits outweigh the potential risks. However, a societal risk is that "elective modifications" will transition from the realm of theory to that of practice.

5. In what cases would you consider germline engineering ethically acceptable?

Moreno: There would have to be a favorable risk/benefit balance for particular cases as well as monitoring of the children, perhaps for a lifetime. More pro-actively as a matter of justice many would require that there be full access to the "advantages" of modifications for all prospective parents, otherwise genetic benefits are locked in to one's descendents (shades of HG Wells' "Time Machine" -- the Warlocks and the Eloi!). But that would seem to require equal access to ART, which is far from the case anywhere at present.

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.

I would even like to raise ethical concerns for a wide application of germline editing in large mammals and not only in humans, at least within the scope of curiosity-driven research. Whereas there would be several potential benefits of germline gene disruption and editing in large animals we should not be unquestionably open to such endeavors. Traditionally, large animals carrying disease-causing mutations have been sought in nature and then bred to allow investigation of the disease pathogenesis and pre-clinical testing of new therapies. These have been extremely valuable models and, because the mutation originated spontaneously in nature and the research aimed at better understanding or treating the disease, there has been a general consensus that these types of studies are ethically acceptable. We can now easily generate a knock-out potentially for any gene in large animals (as mentioned above germline gene disruption is well feasible), thus easing the generation of new disease models and well serving translational research. We should however exercise some caution in broadening such research, given its potential but intended outcome of inflicting severe illness and pain to large animals. There should be strict boundaries on such research with a strong emphasis on its substantial and immediate value for a translational study. On the other hand, curiosity-driven research of potential great scientific interest, such as screening by disruption genes potentially involved in higher brain function, may raises more concerns and should be carefully assessed. As mentioned above, broad use of this strategy may generate societal backlash (and rightly so, in my view).

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"

Corn: I'm not a bioethicist by any means. But speaking for myself, I personally find the potential for permanent cures for debilitating genetic diseases (especially childhood onset) quite exciting. Imagine a severely impacted child knowing they will have problems their whole life and potentially pass their own disorder on to their own children, instead leading a healthy life.

Nature Biotechnology: doi:10.1038/nbt.3227

Charo: It isn't possible to answer this question without information about risks and possible benefits. On both scores, the science is still too early to answer these questions.

Cohen: Specific disease targeting.

Li: The only case of germline engineering ethically acceptable, I think, is used for treatment of human genetic diseases.

Ji: Gene editing in the human germline should only be done once scientists, medical practitioners and ethicists have understood and considered all of the biological and ethical consequences of this approach. In my view, experiments in non-human primates will be necessary to advance knowledge in this respect.

Charpentier: I believe the European convention 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.

Kim: Parents with a homozygous mutation that causes a fatal disease may wish to use germline engineering to avoid transfering their faulty genes to their children. Even parents with a heterozygous dominant mutation would want to eliminate the potential risk, if safe and efficient methods of human germline editing were available. Many parents (most likely mothers who carry an X-linked mutation) with a fatal genetic mutation who have lost a child before would take a risk to correct the genetic defect in germ cells or embryos. Is it moral to illegalize their desperate desire?

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 instance 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 ICSI (intracytoplasmic sperm injection) to have children, not just 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 the methods to confer disease resistance may be considered OK: 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? However, 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 generation having a choice (except the same technology could be used to reverse the enhancement).

Doudna, Carroll, Martin, Botchan:

This is a matter for discussion. We don't know what the best specific candidates for germ line modification are, and we welcome discussions with clinicians and human geneticists.

At a minimum, the engineering maneuver should be considered only to prevent a devastating condition, and in cases where there is no acceptable equivalent alternative.

Bredenoord: Translating germ-line 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 germ-line engineering.

1. Requirement of safety

The process of translating basic research into clinical applications that could potentially lead to larger clinical trials and implementation in the health care system begins with preclinical research and subsequently moves into first-in-man (or phase I) studies. First in man use is ethically challenging by nature, particularly because the needed evidence to reliably predict risk and benefit (testing in humans) is missing. First-in-man germ-line engineering trials will be replete with uncertainties and safety concerns. This needs careful, long-term, interdisciplinary research and sufficient evidence to make the leap from bench to bedside. This by the way also needs more ethics research: while research ethics has made several efforts to better map the risks and benefits of medical research, less work has been done to determine when an acceptable risk-benefit balance has been reached. The appraisal of risks and benefits involves much intuition and much depends on one's attitude towards risk (see also Bredenoord and Braude, Ethics of mitochondrial gene replacement: from bench to bedside. *British Medical Journal 2010;* 341:c6021).

2. Right to an open future

One of the most prominent (non-safety) objections against germ-line 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 think this is the reason that germ-line modification of the nuclear DNA has led to more ethical controversy than modification of the mtDNA.

I have argued earlier (Bredenoord et al, "Ethics of modifying the mitochondrial genome" *Journal of Medical Ethics* 2011;37:97-100) that a clinical application of germ-line 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 towards 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 of which we can assume that they 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.

Zhang: In contrast to germ line engineering, genome editing technologies also have the potential for treating diseases through modification of somatic cells in patients. If we can become successful at developing effective genome editing technologies for somatic cell therapies then we would not need to edit the germ line.

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

Perry: Human germline engineering could be ethically acceptable when it is agreed that the procedure will alleviate the potential for human suffering and is prescriptive above a certain agreed minimum. The thorny nature of the challenges of defining 'acceptable' and 'minimum' are probably not without precedents in clinical medicine (eg prioritisation of resources, euthanasia, gestation time limits on abortion, etc.).

Pera: I think that using the technology to eliminate known deleterious elements with significant impact of the life of the offspring should certainly be considered within the scope of ethically acceptable interventions.

Lanphier: For therapeutic uses only. However, it is difficult to think of a therapeutic application that either cannot currently be addressed by available techniques, such as carrier or pre-implantation screening, or for which the perceived overall benefits outweigh the potential risks.

6. What do you consider the optimal approach to allow necessary research on human germ cells to go forward while overseeing germline clinical applications of CRISPR-Cas9 mediated research engineering? Full international ban, temporary moratorium, regulation or laissez faire?

Moreno: As to the moratorium, I'm unconvinced. It seems to me that for such an action there would need to be the equivalent of a clear and present danger. I don't see that in this case.

Naldini: It is interesting that the type of genetic editing made possible by the CRISPR-Cas9 technology was for the largest part already available for quite a long time by using the ZFN / TALEN platforms. The main difference is the ease by which a lab can today generate an effective reagent to target its gene of interest, much less the efficacy or specificity. Thus, one wonders what has triggered now, rather than earlier, such concerns. There is certainly the need to push forward with research on human ES cells, including gene editing that may serves a large number of applications before germ-line transmission.

Greely: Research should go forward but there should be no efforts to make babies this way pending both research results and a social decision, in different jurisdictions, to allow it, regulate it, or ban it. Personally, I'd probably take the "regulate it" option.

Corn: We are asking for a temporary moratorium on human germline editing research while a wider discussion among representative stakeholders from a variety of areas is underway. We are in the process of initiating a larger meeting for just such a purpose.

Nature Biotechnology: doi:10.1038/nbt.3227

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 US there are some variations in state laws that are relevant. This is why an initial step involves public discussion and development of principles to guide the research.

Cohen: Regulation, as with other medical technologies. Bans and moratoria will only serve to drive the activity underground.

Li: I think the research can be undertaken to test the possibility of correction of genetic defects in human SSCs.

Cohen: Regulation, as with other medical technologies. Bans and moratoria will only serve to drive the activity underground. An international set of standards will help. A teenager, David Hahn, has already built a nuclear reactor in his backyard, using available materials—this has not obviated the utility of international atomic energy and non-proliferation accords

Charpentier: CRISPR-Cas9 has proven to be a very powerful gene editing technology, raising concerns of deviation of its usage for wrong purposes. Consequently, scientists, clinicians, the industry and patients including experts on ethical and related legal questions need to have an open dialogue on the risks and benefits of precise gene editing technologies in germline modification.

Ji: I think we should call for a wide discussion, including all orders of society, instead of moratorium or anything else. And meanwhile tightly control the experiments regarding engineering of the human germline. At same time experiments to elucidate the risks of genome editing in germ cells should be done in non-human primates. We also need to establish a community to strengthen the exchanges and cooperation between scientists.

Kim: Research should be encouraged under certain guidelines rather than banned.

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 its future application in curing some severe diseases.

Lovell-Badge: We are fortunate in the UK to have robust regulation via the Human Fertilization and Embryology Authority (HFEA). This will prevent the use of the methods (which are illegal here, and would require a change in the Human Fertilization and Embryology Act to be voted by UK Parliament) until there is a good degree of confidence that they are likely to be safe and efficient, there has been a proper debate about the potential uses and limits, and a good measure of public acceptance, and how children born using the methods will be subject to follow-up. It is a pity that many other countries lack this type of regulation. There should not be a ban on research – the techniques are too important and could lead to much better understanding of aspects of early human development and to indirect ways of avoiding or treating disease.

Doudna, Carroll, Martin, Botchan

1. 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.
2. A broad discussion of the prospects and limitations will have two positive effects: it will alert people broadly to the concerns about the current technology and potential long-term effects, and 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.

Bredenoord: I do not see why a full international ban would be necessary, for the benefits of germ-line modification may in the future outweigh the disadvantages. I think a laissez faire approach is no option at all, due to the safety concerns and societal risks. I would therefore opt for either a temporary moratorium, or regulation (like in novel pharmaceuticals).

Zhang: Similar to the early days of research with in vitro fertilization and embryonic stem cells, we should allow careful and ethical use of human germ cells for scientific research at the cellular level. Strict guidelines should be put in place. Temporary moratorium while we figure out important regulatory guidelines is appropriate.

Feng: I think that we should have a temporary moratorium until all key technical issues are solved, international regulatory guidelines are established and monitoring systems are in place. This is not something we can take it lightly.

Perry: It's my understanding that in the UK, 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 and even if it were it's unclear to me how it would be policed. This debate cannot be seen in isolation: for example, the China would be less inclined to listen to the US 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 (see below) 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. Have such clarifications been made? 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.

Nature Biotechnology: doi:10.1038/nbt.3227

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.

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.

Rosario Isasi & Bartha Knoppers: Enacting or promoting policy action based only on safety and efficacy considerations represents a necessary but limited approach. Undoubtedly, safety and quality requirements are essential pre-requisites for scientific integrity; but like temporary bans or moratoria, they are time-limited. Once hurdles relating to safety and efficiency issues are resolved, the fundamental question remains unanswered: If citizens contribute to providing some direction for science, how far to go? What are the ethical thresholds? Caution and deliberation allows the construction of sufficient tools to assess (and continuously re-assess) the ethical issues. Shifting the burden of proof without allowing the gathering of robust evidence on the benefits and risks of any technology is not a justifiable approach.

7. Is it possible to have an Asilomar-type resolution today given the questions swirling around CRISPR engineering of the germline and the international nature of research, the number of countries involved and ease of technology/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.

Naldini: It will be certainly useful.

Greely: Well, Asilomar wasn't a "resolution" but one step in a process that led to a resolution. I think it is possible to have a similarly useful step - especially since there's no great crying need or demand for human germ line genomic modification.

Corn: Scientists are people, like anyone else. There's no accounting for a few bad actors, but I think is a national or international community raises concerns about germline engineering, I think that for the most part people would respect that. Certainly there wouldn't be a widespread effort towards implementation while significant concerns were outstanding. As for garage biology, I think this is an exciting development but doesn't really impact human germline engineering. As mentioned above, the bottlenecks have moved. People may be using CRISPR in their garages, but it's mostly in model systems that are garage-tractable. Even if CRISPR is easy enough for the garage, maintaining relevant human cells and doing the downstream work is not.

Nature Biotechnology: doi:10.1038/nbt.3227

Charpentier: I am relatively optimistic that based on an extensive and transparent dialogue one could achieve a resolution where scientists, clinicians, and the industry around the world may commit to.

Ji: I think Asilomar-type resolution is a good solution to deal with the questions swirling around CRISPR engineering of the germline.

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. I am a lot more concerned about Cas9-mediated gene drive (recently reported in *Science*) in animals and plants. An organism with a Cas9 gene could be created in a garage and released to the environment. This may trigger unexpected ecological outcomes.

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.

Lovell-Badge: I am on the organizing committee of the Hinxton Group (see: http://www.hinxtongroup.org), which will have a meeting in September this year on this topic. This will bring together international experts in the methods, other relevant sectors of the science community, ethics, sociology, etc. It is not possible to anticipate the outcome, but I hope that this will come up with a set of recommendations that could be followed.

Doudna, Carroll, Martin, Botchan

1. It's not clear that the Asilomar guidelines governed all recombinant DNA research around the world. Fortunately, there were few, if any, real hazards
2. Establishing guidelines for CRISPR engineering of the germ line will have an influence on the vast majority of people.

Bredenoord: Regulation should be drafted on both the international and national levels. The challenge of international regulation and organizations such as the WHO is the difficulties in imposing sanctions. I would therefore also favor national regulations (also due to changes in political and societal cultures). Moreover, international professional societies should take up their responsibilities (such as the International Society for stem Cell Research and various genetics associations). Also journal editors play an important role here, as they can set the quality standards. Also Research Ethics Committees and oversight should be enhanced throughout the world.

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 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

Nature Biotechnology: doi:10.1038/nbt.3227

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 dialogue 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.

Zhang: It is important to organize broad range of experts to carry out thoughtful discussions and brainstorms to craft strategies to regulate but still enable research advances. We also need to anticipate all problems that might arise in the future and plan strategies. It is important to be proactive rather than reactive.

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: This seems unlikely. One has to compare the circumstances surrounding Asilomar and the human germline debate. Then, 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 may be 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. Given this, a moratorium is at worst neutral; what's not to like? 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; what's not to dislike?

Two general points are also related to this question. First, the US probably does not hold the same sway as it did in 1975. The second point comes by way of precedent. Given the considerable lag between false claim to have generated human ntES 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 last 15 years but turned out to be nothing more than 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.

Pera: It is definitely possible to have an Asilomar type resolution on human germ line modification that provides for a cautious and reasoned approach. Implementation of germ line genetic modification in the human is most unlikely to take place in the context of garage biology. There were endless warnings about how humans would be cloned once the technology was available. Nothing like that happened, firstly because such interventions require large teams of people with appropriate medical and scientific expertise and facilities, but more importantly,

because the scientific community behaved in a reasonable and responsible fashion.

Lanphier: It's uncertain that a similarly impactful resolution could be generated today, however we need to try. A strong consensus from such a meeting could have a meaningful impact on future research

Isasi & Knoppers: Are we ready for a revamped (and more inclusive) 'moratorium' version of Asilomar? Or for an "actionable" international treaty? Given competing agendas and vested interests, what would a coherent approach to a consensual policy look like? 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 non-germline 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 inducing genome modification based on fears over slippery slopes resulting in eugenic scenarios.

J. Craig 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 the 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.

8. Does the fact that CRISPR technology works relatively easily in different labs across the world impact on the effectiveness of a ban or moratorium. Does the high adoption and reproducibility of CRISPRs make it different from germline gene therapy or reproductive cloning?

Naldini: As mentioned above, there are still significant technical hurdles to attain genome editing (except for gene disruption) in embryos for reproductive purposes.

Greely: Of course that 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: As mentioned above, 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, even for things like somatic (e.g. hematopoietic) editing.

Nature Biotechnology: doi:10.1038/nbt.3227

Ji: As CRISPR technology is easy to repeat, even very small labs can conduct these types of experiments. This does make it different from traditional gene therapy or reproductive cloning. The characteristics of this technology make it difficult to ban or suspend.

Kim: I don't think that CRISPR is any different from cloning or germline gene therapy with regard to regulation and ethics. Transgenic humans who express green fluorescent protein, for example, have never been created in clinics not because it is technically difficult but because it is illegal and morally unacceptable.

Zhou: The CRISPR technology does make the germline modification more accessible, and this may impact the effectiveness of a ban or moratorium to some extent. However, I think the main risks and ethical controversies are the same and not dependent on which genome-editing technology adopted.

Lovell-Badge: The CRISPR technology can be used as a form of germline gene therapy, but with wider implications. Its efficiency and relative ease of use will make it hard to control. Reproductive cloning is different, in part because all the experience gained in animals says that it is unsafe: the majority of attempts fail as early embryos, during gestation, or postnatally, or animals that do live often develop problems later on. However, in my view, there is also no good reason for carrying out reproductive cloning in humans.

Doudna, Carroll, Martin & Botchan:

1. The precision of CRISPR edits makes it different from germ line gene therapy by "conventional" methods.
2. CRISPR editing could be done in conjunction with reproductive cloning by somatic cell nuclear transfer.

Bredenoord: As said above: new biomedical technologies always impact society. The easier a novel technique is applicable and affordable, the more rapid and extensive the societal impacts will be. If there are less "technical" barriers and constraints for people to use a technique, people should make an appeal to ethical standards and personal morality in order to determine whether a technique is ethically defendable and a particular use is acceptable.

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 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 I see it, there are two embodiments of genome editing at present. In one, a double-strand break is made at a defined genomic position (by TALENs, ZFNs or Cas9) and repaired by NHEJ in a cut-and-paste mechanism. This results in small, as-yet unpredictable indels and for this reason - because there is an inherent element of unpredictability - I see no application of this to the human germline. On the other hand, the double-strand DNA break can be fixed via the HDR pathway - homology-directed repair - and this is what would likely be harnessed in the future, (assuming either technology is), so that precisely prescribed human genomic changes can be introduced. The differences between NHEJ and HDR pathways are an important facet of the debate. 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.

Lanphier: 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.

9. The UK recently approved mitochondrial replacement therapy and human somatic cell nuclear transfer into an enucleated oocyte has been recently successfully achieved in vitro. Both techniques involve germline changes to the human genome, and researchers involved in these types of studies could help inform the debate. How different are the ethical challenges posed by CRISPR germline engineering?

Moreno: Mitochondrial replacement has much more limited and less objectionable results and SCNT is very cumbersome. CRISPR, TALEN etc. seem to leave them in the dust in just about every way, if they are as described in the literature.

Naldini: Reproductive cloning raises the highest ethical concerns, as it abrogates the unique identity of the self, and reduces the progeny to an object made at an individual's will and purpose; while within a closer reach than gene editing it is and should be banned. Mitochondrial replacement therapy deals with a very small portion of genetic inheritance, which is loosely connected with the identity of the self, and does not introduce novel or artificial sequences in humans, thus being conceivably ethically acceptable. Gene editing, albeit dealing with the "main" line of germline transmission, also target a very small portion of it, with the potential, however, to alter in an unprecedented manner the human gene pool. As discussed above, once feasible and safe, germ-line editing may become ethically acceptable for in situ gene correction of inherited mutations to the wild type version.

Somatic nuclear transfer into oocytes to generate syngeneic ES cells for somatic tissue replacement should become acceptable to most cultural and religious backgrounds.

Greely: Significantly different than mitochondrial replacement because a) the safety risks of CRISPR seem higher and less clear, and b) the ability to modify any specific piece of the inherited DNA, as opposed to only the mt DNA, seems significant in terms of safety and social

impact. Somatic cell nuclear transfer – the "Dolly" cloning technique – continues to have very large safety issues and it is not clear whether anyone could ever make a compelling case that it was necessary, or even worthwhile.

Corn: Mitochondrial replacement is very exciting! I think the difference is that CRISPR engineering allows us to make deliberate, reductionist changes instead of transplanting an existing system. That's an exciting opportunity, but do we understand enough about human genetics to make those changes with full knowledge of all the downstream consequences?

Cohen: Nuclear/mitochondrial transfer is more laborious and requires more expensive and refined equipment. It also does not have the same potential scope of applications as CRISPR. The ethical challenges of CRISPR are magnified by its far larger potential accessibility and practice, as well as by the far greater scope of change to the genome.

Li: The two techniques you mentioned actually have been approved for the application of human disease treatment. Similarly, if CRISPR-Cas9 applications in germ cells will be adopted for genetic correction, it is also acceptable.

Charpentier: The concept is at least closely related. It will be certainly helpful to involve researchers who already have dealt with mitochondrial replacement therapy.

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.

Kim: I do not see much difference between the two in this issue.

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

1. 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.
2. No nuclease-based engineering is involved, so there will be no off-target mutagenesis.
3. Nonetheless, mitochondrial transfer is permanent, and the same issues of unpredicted effects of novel alleles in a given background mentioned above apply.
4. It is also true that, unlike the nuclear genome, deleterious effects in transplanted mitochondria cannot be moderated by sexual reproduction, since the organelle is inherited uniparentally.

Nature Biotechnology: doi:10.1038/nbt.3227

Brendenoord: See also my earlier answers: many ethical issues overlap, but the scale is different: mtDNA mutations are less prevalent, and mitochondrial gene transfer will not be used on a massive scale. Gene editing has the potential to be a "game-changer" in biomedicine.

Bosley: The UK'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 a number of technical reasons, its implications are much more constrained than the CRISPR/Cas9 technology. With the mitochondrial replacement approaches, 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 judgement can be made about whether that balance is acceptable. And the UK made that judgement 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.

Zhang: Although there are similar challenges between mitochondrial transplantation and germ line genome editing, the main difference is that mitochondrial transplantation does not attempt to make any artificial/unnatural changes to the cell. The mitochondria is intact and is not necessarily different than the mitochondria received through natural fertilization. However, germ line genome editing introduces something that is artificial.

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.

Perry: Mitochondrial replacement and nuclear transfer are different in principle 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. 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 pre-existing 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 ntES cells are also of limited relevance to discussions about human germline genome engineering.

Pera: The main differences are first that mitochondrial replacement is a relatively well circumscribed intervention in terms of its application, and second that the consequences of passing on these disorders are devastating.

Nature Biotechnology: doi:10.1038/nbt.3227

Lanphier: Both processes are therapeutic applications that involve “global” or “wholesale” transfer of pre-existing genetic material rather than the targeted alteration of specific genes. It is less evident that these technologies could be used for eugenic or “elective” purposes.

10. Is international oversight necessary/possible or would national oversight suffice? Who do you consider the correct regulatory/government agencies to oversee this research? (NIH’s RAC (now disbanded), medical associations? European Union?)

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 GOF [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 US is not a part of the International Criminal Court, for example. With regard to some potential abuses -- apart from germline modification, can gene editing be exploited for more efficient research on biological weapons? -- one could look to the Biologic and Toxin Weapons Convention. The next five-year review process for the BTWC is December 2016. In light of the trajectory of genetic editing techniques they should be on the table at the review.

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 stakeholders should suffice upon informed advice by scientific societies/representatives. Scientific societies and communities should hold a debate and express general recommendations.

Greely: The organization depends in part on the mission, which remains undefined. I wouldn't expect anything international to have real enforcement teeth, but it could prove useful; I suspect, though, that whatever is done will largely be national (or, in the case of the EU, regional).

Corn: I could imagine multiple organizations potentially getting involved, but this question is exactly what we hope to address at the upcoming, larger meeting on germline engineering.

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

Nature Biotechnology: doi:10.1038/nbt.3227

Ji: I think one would need special government agencies in the respective countries to oversee this research is conducted properly.

Kim: International oversight would be difficult to implement. Perhaps, an international organization could provide advice to member countries, and each country would then decide how to regulate germline genome editing and who would oversee this work within its borders.

Zhou: I would prefer "international guidelines plus national oversight policies" to oversee human germline engineering. The medical services, academic institutions and industries face the same scientific and technical barriers, but the ethical challenges are different in different countries due to differences in society, religions, economics, *et al.* 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.

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 listened to by clinicians.

Doudna, Carroll, Martin & Botchan:

1. RAC has not been disbanded. It has meetings scheduled in June, September and December this year.
2. RAC now reviews all proposals for gene therapy, including ones using designer nucleases (no CRISPR protocols have been submitted, as far as we know).
3. FDA also reviews such proposals, since genes and nucleases are viewed as drugs.
4. There needs to be national regulatory review in the USA. It would be good to have agreed-upon standards internationally.

Bredenoord: I think both international and national regulation and oversight is necessary. In addition, professional societies and researchers themselves should take their responsibility. Also journal editors play an important role here. See also sub 7.

Zhang: These are very challenging issues and we should begin with education of as many people as possible so that they there is accurate understanding of genome editing and its scientific, societal, and ethical implications. Concurrently, a group of experts consisting of scientific, technical, ethical, and policy thought leaders should be convened to identify the best path forward.

Feng: Should have both national and international oversight.

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 neutralise 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 non-editing 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.

Pera: There is no one model that will fit all jurisdictions. The pre-regulatory discussion phase, which is where we should be during the moratorium, should engage scientific and medical societies, legal and ethical experts, patient advisory groups, and lay members of the public.

Lanphier: International oversight may not be effective or easily enforced. However, for applications regarding the use of germline genetic engineering for therapeutic purposes to treat genetic disease in the United States, the relevant regulatory body would be the Center for Biologics Evaluation and Research of the U.S. FDA. Additional oversight could be provided by Institutional Review Boards of hospitals where the procedure would take place. Incidentally, the RAC has not been disbanded.