Presidenza del Consiglio dei Ministri

ETHICAL ISSUES IN GENOME EDITING USING CRISPR/CAS9

23 February 2017

INDEX

Presentation

The opinion analyzes the CRISP-Cas9 technique within the context of recent, highly innovative techniques in genetic engineering, which can modify the DNA sequences of living organisms with high precision, relative ease and low cost.

Following a scientific description of the *gene-editing* technique, the document focuses on the peculiarities of the technique, its potentialities, risks and possible applications in the context of the emerging current bioethical debate in the scientific sphere.

From the discussion of the Committee there emerge some common elements as well as divergence of opinion.

The Committee is in favour of in vitro and animal testing, in accordance with international rules, in order to test the safety and efficacy of technologies and considers it ethically desirable to increase research on human somatic cells both in laboratory research as well as in clinical or in-vivo research.

As far as *gene editing* on the human germ line is concerned, it does not consider testing on gametes, intended for conception, and human embryos to be implanted, to be legitimate, agreeing on the opportunity for the moratorium on clinical research or in-vivo research, until the necessary conditions of safety and efficacy of the technique have been achieved.

The Committee expresses opposite viewpoints on experimentation regarding *gene editing* in the lab on gametes not intended for reproduction and on in vitro embryos not intended for implantation: some are in favour while others are against on the basis of opposing arguments.

A brief history of genetic engineering is provided in the Appendix to outline the context of the birth of *gene editing* and an analysis of key international documents as well as national regulation.

The working group was set up on November 19, 2015 and the work was progressively coordinated by Profs. Stefano Canestrari, Carlo Casonato, Bruno Dallapiccola, Assuntina Morresi, Demetrio Neri, Laura Palazzani, Monica Toraldo di Francia, Grazia Zuffa.

Prof. Demetrio Neri on April 25, 2016 resigned from the National Committee for Bioethics.

Several drafts were produced and discussed at plenary sessions. Having failed to elaborate a draft document that could find consensus in the Assembly, the Deputy President Prof. Lorenzo d'Avack reworked the text, which was approved in the plenary session of 23 February 2017 by Profs.: Salvatore Amato, Luciano Battaglia, Stefano Canestrari, Cinzia Caporale, Carlo Casonato, Antonio Da Re, Lorenzo d'Avack, Mario de Curtis, Riccardo Di Segni, Assuntina Morresi, Andrea Nicolussi, Laura Palazzani, Monica Toraldo di Francia, Grazia Zuffa.

Professor Carlo Flamigni abstained.

The advisory members Dott. Maurizio Benato (FNOMCEO) and Dott. Anna Teresa Palamara (CSS), Carlo Petrini (ISS) voted in favour.

Profs: Carlo Caltagirone, Francesco D'Agostino, Bruno Dallapiccola, Paola Frati, Silvio Garattini, Marianna Gensabella, Rodolfo Proietti, Massimo Sargiacomo, Lucetta Scaraffia and the advisory member, Dott Carla Bernasconi (FNOVI) absent from the plenary session, subsequently gave their support.

Personal remarks were later received from Profs. Carlo Flamigni and Assuntina Morresi and Bruno Dallapiccola (the latter was adhered to and signed also by Prof. Francesco D'Agostino).

Important contributions were made by the hearings of Prof. Luigi Naldini, Director of the San Raffaele Telethon Institute and Professor of Histology and Cell and Gene Therapy at the University Vita-Salute San Raffaele in Milan (November 19, 2015) and by Prof. Demetrio Neri, member of the NBC (10 December 2015).

1. Introduction: the CRISPR-Cas9 technique

The debate around genetic engineering and the ethical problems raised by the possibility of modifying the DNA of living, human and non-human organisms dates back to the 1970s when a group of scientists called for a moratorium on recombinant DNA research. The alarm prompted scientists to develop a self-regulatory code to address the security issues underlying the request for the moratorium[1]. It is in this context and within its developments in the medical field that this discussion should be placed.

Definition and description of the technique

Genetic engineering has seen the emergence of highly innovative techniques which can modify the DNA sequences of living organisms with high precision, relative ease and low cost. The commonly used expression, is, *gene editing or genome editing*, terms translatable in Italian with the words modification, correction, genetic or genomic revision. The expression believed to be most appropriate is *gene editing* as the modifications usually relate to a gene sequence.

These are genetic engineering techniques that use "molecular scissors" to cut DNA at precise points in order to eliminate some parts, correct them and/or replace them. An *editing* can find an error in the DNA sequence, even a single base, correct it and restore the wild sequence[2]. It is in this context that CRISPR-Cas9 (*Clustered Regularly Interspaced Short Palindromic Repeats*) has been developed, a technical acronym that indicates a brief sequence of RNA (ribonucleic acid) constructed in the laboratory, designed to identify a precise genome region and guide the Cas9 enzyme (belonging to the restriction nuclease group), a kind of "biological scissor" able to cut DNA in the region selected by the researcher. The part of the DNA removed, as "defective", can be deleted or replaced with a "normal" sequence.

The novelty lies not so much in the idea, but rather in the molecular assembly designed to perform the *editing* operation, which opens prospects for intervention unimaginable until a few years ago, with characteristics of precision, specificity, relative simplicity, easy accessibility, efficiency and low costs.

The peculiarity of the technique: potentialities and risks

Genetic information contained in DNA allows the synthesis of all the proteins necessary for the life of all organisms. The ability to selectively modify the genome of any living being, as offered by *gene editing* in the CRISPR-Cas9 mode, allows for relatively easy study of the function of genes (i.e. their biological activity) and the elements that regulate them: with these new techniques it is therefore possible to study the structural organization of the genome of any organism, by modifying the sequences and, therefore, the functions of DNA in the context of the genome, monitoring the results of the changes within the cell or organism.

[1] The self-regulatory code was developed at the conference held in Asilomar (February 1975).
[2] Wild gene sequence means primitive form of an allele, as a rule the most common form in the population.

This peculiarity is very important in view of the complexity of the genome: an error, even of modest magnitude, induced by genetic modification, can alter not only the structure of the coded protein but also block its production, with unforeseeable consequences for the organism.

The CRISPR-Cas9 technique is at present the subject of great attention by researchers in the field, in view of the potential both in terms of improvement of basic knowledge of cellular development processes, as well as of possible applications to a series of sectors related to agriculture, breeding, industrial biotechnology and, in general, life sciences and technologies applied to the human being. International scientific literature, in this respect, shows a particular interest in medical research, the development of drugs, basic biology, and the particularly sensitive sector of human reproduction.

However, it should be remembered that only 2% of the human genome encodes for proteins (i.e. is directly involved in the production of proteins). By modifying this 2% using *gene editing*, it is possible to correct gene-disease or build disease patterns, even sequentially modifying multiple genes acting in an additive manner to give rise to complex diseases (in practice, it is possible to modify the genetic inheritance of the cells in vitro, i.e. in a laboratory, or in vivo, that is, in animal models, by introducing the genetic characteristics of hereditary diseases). Gene editing can also be applied to 98% of the human genome that does not encode for protein, once improperly defined as "junk DNA", which we know has important regulatory functions.

Given that, as has been said, the idea of introducing controlled alterations in the genome is not new, there is the preliminary question of how to consider this innovative technique. It can be seen as a new tool better than the ones already available, but that does not change the overall picture of genome modification and its prospects; or as a tool with the transformational potential to radically reconfigure expectations and ambitions with regard to science and its application capabilities[3].

Embracing the first or second perspective may have an impact on the framing and the urgency of public debate. This discussion must consider both the potential of the technique and the possible current risks associated with the complexity of these interventions on the human genome. Many authoritative scientific journals have broadly debated these issues, for example. "Science"[4], "Nature"[5], "Cell"[6], and some popular magazines[7].

Potential applications

As mentioned earlier, *gene editing* techniques have great cognitive potential and variegated applications: in the non-human field, from animal breeding to industrial biotechnology, from the biosphere to plants; in the human sphere, from enhancing reproduction-related knowledge to clinical applications.

[3] On the opportunities and framework of the public debate on the most recent developments *in genome-editing*, see: *Public dialogue on genome editing. Why? When? Who?* Report of a Workshop on Public Dialogue for Genomediting, Nuffield Council on Bioethics, May 2016.

[4] http://www.sciencemag.org/topic/crispr.

[5] http://www.nature.com/news/crispr-1.17547.

[6] http://www.cell.com/nucleus-CRISPR.

[7] Like: *DNA Revolution*, in “National Geographic”, August 2016, e “The Economist”, www.economist.com/news/leaders/21661651-new-technique-manipulating-genes-holds-great-promisebut-rules-are-needed-govern-its.

One of the most discussed issues in the bioethics debate regards intervention on humans: on somatic cells, germ cells (gametes), embryos at early stages of development.

Particular attention was paid to the possible implications of hereditary genome modifications and the prospects for immediate and future gene therapy, which raise numerous issues. With regard to prospects linked to better knowledge of embryonic development and applications in the field of human health (such as cellular and xenograft therapies, for example) there are some problems, such as those relating to the safety and efficacy of genetic therapeutic intervention, made even more difficult by the difficulty of calculating the risk-benefit ratio in a field where much remains to be discovered; from the not simple identification of proper therapeutic uses, at a phase of medicine in which, with the development of its preventive orientation, the boundary between curative purposes and *enhancement* tends to become blurred; to questions about the responsibility for the generations to come for the modifications that in future could be introduced, intentionally and not intentionally, into the human hereditary genome. The special attention given, in the debate, to inherited modifications, that is to those induced in embryos at early stages of development and in the gametes used for reproductive purposes, is explained, on the one hand, by the delicacy of the ethical issues involved, and on the other by the approaching unprecedented prospect of being able to undertake genetic correction on gametes or embryos, in order to prevent the birth of children with serious genetic disorders.

In the international debate, as will be seen in paragraph 2, *gene editing* on the germ line is the modification of the DNA of the nucleus of reproductive cells that transmits information from one generation to the next: by modifying the genome of fertilized eggs and embryos, the genetic *make up* of each differentiated cell in an organism is altered, with the result of transferring the change to the progeny.

In this opinion, however, in the section concerning the NBC's reflections, reference is made to the expression germline, specifically to indicate germ cells, that is, male and female gametes, whereas the term "embryos" refers, in particular, to viable in vitro embryos.

2. The bioethical debate and scientific research

As part of the resumption of debate on *gene editing*, the theme of the implications of modification of the human genome began to be discussed in more detail by the scientific community on January 24, 2015 *IGI Bioethics Forum*, in Napa, California, which addressed the scientific, medical and legal implications and ethical implications of this new perspective. The aim was to launch an informed discussion on the use of genetic engineering and to identify the areas of action in view of potential future developments. That meeting outlined some recommendations aimed at ensuring that the technique was applied in compliance with ethical and security principles[8].

The public debate on *gene editing* exploded from two letters. The first, published in the journal "Nature" on March 12, 2015 ("*Do not edit the human*

[8] P. Liang et al., *CRISPR/Cas9 Mediated Gene Editing in Human Tripronuclear Zygote*, in & quot; Protein Cell & quot; 2015 May, 6 (5), pp. 363-372, www.ncbi.nim.gov/pmc/articles/PMC4417674/.

germ line")[9], the second published in the journal "Science" on 19 March 2015 ("*A prudent path forward for genomic engineering and germline gene modification. A framework for open discourse on the use of CRISPR-Cas9 technology to manipulate the human genome is urgently needed*")[10], signed by several Nobel laureates and internationally renowned scholars, directly involved in these technologies. The authors of these letters, with different arguments and with a different perspective, called for a moratorium on the clinical application of the new technique to cells in the human germ line.

For those subscribing to the letter published in "Nature", the call for moratorium should also extend to basic and preclinical research because "we can not imagine a situation where its use in human embryos could offer therapeutic benefit beyond the methods already existing. It would be difficult to check exactly how many cells are modified." Different, although not new, are the motivations put forward by the promoters of the letter (all involved in the development of somatic gene therapy) for the ban on gene therapy research on the germline:

- the desire to clarify to the public the distinction between *gene editing* in somatic cells and germ cells, so that possible social alarm does not discourage the development of somatic gene therapy;
- the inability to know the effects of genetic modification on an embryo until after birth and beyond;
- the possibility that germline research can be exploited to induce non-therapeutic modifications;
- the rarity of the clinical indications of these techniques in their applications to the human embryo and in any case of the dubious lawfulness of the ability to modify the genome in the absence of "consensus" of future generations.

For those subscribing to the letter in "Science", on the one hand the moratorium on the clinical applications of the new technique on the human embryo should be maintained and public reflection promoted on the "new era" of biology and the social, environmental and ethical implications of new techniques[11], while on the other research "to evaluate the efficacy and specificity of CRISPR-Cas 9 technology in human and non-human models relevant to potential germ line germ line therapy" should be encouraged. This research is considered essential to be able to decide on any future clinical applications.

As you can see, the two letters raise many questions of a different nature: from those strictly scientific, relating to gene therapy on germline and the necessary efficacy and safety levels, and moving on, from basic experimentation (on animals and human gametes or on embryos not intended for implantation), to clinical research (on embryos intended for implantation), to strictly ethical questions, on the moral legitimacy of intentionally modifying the hereditary genetic line.

The two letters were published as confidential information was circulating about the imminent disclosure of results by Chinese researchers who had

[9] E. Lanphier et al., *Do Not Edit the Human Germ Line*, in "Nature", 2015, vol. 519, No. 7544, www.nature.com/news/don-t-edit-the-human-germ-line-1.7111.

[10] D. Baltimore et al. Al., *A Prudent Pathway for Genomic Engineering and Germline Gene Modification. A framework for Open Discourse on the Use of CRISPR-Cas9 Technology to Manipulate the Human Genome is Urgently Needed,* in "Science", vol. 348, No. 6230, p. 36-38, www.uam.es/personal_pdi/ciencias/jmsierra/documents/Baltimore2015Sci.pdf.

[11] Resuming issues and problems already present in the discussion at the Napa conference.

applied the new *gene editing* technique to non-implantable human embryos. Of the two articles so far published about this application, the first, announced, is dated April 18, 2015 and is from the magazine "Protein Cell": "*CRISPR / Cas9 mediated gene editing in human tripronuclear zygote*".

In summary, 86 human embryos with anomalies that would have prevented their development, bearing beta-thalassemia, were subjected to *gene editing* to correct the gene responsible for hematological disease with very few encouraging results[12]. This work has shown that there are still many obstacles to overcome before *editing* on the germline becomes feasible. The editorial of the magazine stressed that publishing the study with its negative results was done as a public service. The authoritative magazines "Nature" and "Science" made it known that they had refused its publication.

On 6 April 2016, a group of researchers from Guangzhou Medical University published the second study on the application of CRISPR-Cas9 on human embryos, with particular reference to a mutation that offers resistance to HIV[13]. Even in this case the results were not satisfactory[14].

In May 2015, some US scientific organizations announced an international summit on the topic, which took place from December 1st to December 3rd in Washington. This *International Summit on human gene editing* was convened by the Chinese Academy of Science, the Royal Society, USA. National Academy of Sciences and the United States National Academy of Medicine[15]. The final statement expressed the need to continue basic and preclinical research in general, specifying that cells from human embryos at the very first stages of development and germ cells subjected to gene editing should not be used for reproductive purposes. By differentiating clinical applications to somatic cells from those on germ cells and human embryos, it has promoted the first mentioned and blocked the others, defining as "irresponsible" any clinical use as long as the problems of efficacy and safety remain unsolved, and a broad societal consensus on the appropriateness of the technique is not reached[16].

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[12] Of the 71 embryos developed up to the 8-cell stage, 54, 28 of which no longer contained the mutated gene, but only in some of them the DNA had been properly altered, as scientists planned. In fact, the technique had introduced other unexpected mutations, with unknown outcomes.

[13] Xiangjin Kang, Wenyin He, Yuling Huang, Qian Yu, Yaoyong Chen, Xingcheng Gao, Xiaofang Sun, Y Yong Fan, *Introducing Precise Genetic Modifications into Human 3PN Embryos by CRISPR/Cas-mediated Genome Editing*, J. Assist. Reprod. Genet, 2016, 33, pp. 581-588.

[14] Of the 213 abnormal (three pronuclear) embryos available for research, 45 were subjected to *gene editing*; 26 developed to the stage of at least 8 cells and only 4 incorporated the desired mutation, which was not verifiable in its effectiveness, as only obtained in a copy of the gene.

[15] Reference site: www.nationalacademies.org/gene-editing/gene_167925.

[16] In particular:

1. Basic and Preclinical Research. Intensive basic and preclinical research is clearly needed and should proceed, subject to appropriate legal and ethical rules and oversight, on (i) technologies for editing genetic sequences in human cells, (ii) the potential benefits and risks of proposed clinical uses, and (iii) understanding the biology of human embryos and germline cells. If, in the process of research, early human embryos or germline cells undergo gene editing, the modified cells should not be used to establish a pregnancy.

2. Clinical Use: Somatic. Many promising and valuable clinical applications of gene editing are directed at altering genetic sequences only in somatic cells – that is, cells whose genomes are not transmitted to the next generation. Examples that have been proposed include editing genes for sickle-cell anemia in blood cells or for improving the ability of immune cells to target cancer. There is a need to understand the risks, such as inaccurate editing, and the potential benefits of each proposed genetic modification. Because proposed clinical uses are intended to affect only the individual who receives them, they can be appropriately and rigorously evaluated

At the same time, the drafters of the conclusions hope that the question of the clinical applicability of *gene editing* on the germline may be regularly reviewed, as scientific knowledge progresses and society evolves. The invitation is addressed to the international community, whose effort is required for the setting of regulatory norms on the conditions of acceptable *editing* on the human germline and harmonization of the various existing regulations in this field. During the summit, it was therefore proposed to create a workgroup for the production of global guidelines on *gene editing*, subsequently elaborated and then presented on February 14, 2017 in Washington. It is a thick volume entitled *Human genome editing: Science, Ethics and Governance*, which addresses different aspects of the applications of *gene editing* on human subjects, from laboratory experiments on somatic cells and germ cells and embryos to possible clinical trials in adults[17].

Meanwhile, human embryo *gene editing* experiments have been authorized at the Karolinska Institute in Stockholm in June 2015[18], by the English HFEA Authority at the Francis Crick Institute in London, February 1, 2016[19], and in Japan on April 22, 2016[20].

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within existing and evolving regulatory frameworks for gene therapy, and regulators can weigh risks and potential benefits in approving clinical trials and therapies.

3. Clinical Use: Germline. Gene editing might also be used, in principle, to make genetic alterations in gametes or embryos, which will be carried by all of the cells of a resulting child and will be passed on to subsequent generations as part of the human gene pool. Examples that have been proposed range from avoidance of severe inherited diseases to 'enhancement' of human capabilities. Such modifications of human genomes might include the introduction of naturally occurring variants or totally novel genetic changes thought to be beneficial. Germline editing poses many important issues, including: (i) the risks of inaccurate editing (such as off-target mutations) and incomplete editing of the cells of early-stage embryos (mosaicism); (ii) the difficulty of predicting harmful effects that genetic changes may have under the wide range of circumstances experienced by the human population, including interactions with other genetic variants and with the environment; (iii) the obligation to consider implications for both the individual and the future generations who will carry the genetic alterations; (iv) the fact that, once introduced into the human population, genetic alterations would be difficult to remove and would not remain within any single community or country; (v) the possibility that permanent genetic 'enhancements' to subsets of the population could exacerbate social inequities or be used coercively; and (vi) the moral and ethical considerations in purposefully altering human evolution using this technology. It would be irresponsible to proceed with any clinical use of germline editing unless and until (i) the relevant safety and efficacy issues have been resolved, based on appropriate understanding and balancing of risks, potential benefits, and alternatives, and (ii) there is broad societal consensus about the appropriateness of the proposed application. Moreover, any clinical use should proceed only under appropriate regulatory oversight.

[17] Committee on Human Gene Editing: Scientific, Medical, and Ethical Considerations, A Report of the National Academy of Sciences and the National Academy of Medicine: Human Genome editing: Science, Ethics and Governance, The National Academies Press, Washington, D.C. 2017, http://www.nap.edu/24623.

[18] www.nature.com/news/gene-editing-research-in-human-embryos-gains-momentum-1.19767.

[19] www.nature.com/news/uk-scientists-gain-licence-to-edit-genes-in-human-embryos-1.19270. It is worth recalling that the English Act from 1990 (*Human Fertilization and Embriology Act*, revised in 2008) allows experimentation on embryos until the 14th day of development, with the clause of their non-transferability in uterus. The HFEA is the Authority to examine and authorize the research protocols of this type of study.

[20]www.japantimes.co.jp/news/2016/04/22/national/science-health/japan-panel-greenlights-gene-editing-of-human-eggs-for-basic-study/#.V1WWYleaBp-.

3. Some preliminary distinctions prior to bioethical reflection

The Committee limits itself to addressing certain areas of use of gene-editing, which however have raised important questions, and it intends to preliminarily distinguish between:

a) studies designed to improve basic knowledge and cellular development processes;

b) genetic interventions designed to modify/repair the DNA that contains a mutation uniquely attributable to a known disease (e.g. cystic fibrosis);

c) other interventions, more or less attributable to an improvement of the phenotype and genetic heritage.

All cases distinguish between:

1. Interventions on germ cells and embryos, which would introduce potentially transmissible modifications to future generations;

2. Interventions on somatic cells of embryos in the uterus[21] or fetuses, or children or adults, where modification may have a therapeutic effect, but would not be inheritable.

Genetic intervention for therapeutic purposes on somatic cells of adults or fetuses (case b.2) presents the same ethical problems of all experimentation on humans, children and fetuses.

Genetic improvement interventions (case c) fit (complicating it) into the debate about the borderline between "therapy" and "enhancement". They are in addition to, in case c.1, the same considerations on embryo testing, as outlined below. This is an extremely interesting issue, which can be made the subject of a specific opinion[22].

This document intends to focus on some specific elements with respect to new *gene editing* technology, in particular on the increase in knowledge and genetic correction for therapeutic purposes, capable of inducing inherited modifications, as well as the expected modifications, for reproductive purposes, to germ cells and embryos in the early stages of development (case b.1), one of the main themes of the international debate.

While not entering into the merits of the issues already discussed in previous documents, the NBC is aware that the discussion on the application of *gene editing* to the germline and embryos is related to the issues raised by the currently available practices to prevent the birth of children with serious genetic diseases (pre-implant or prenatal diagnosis, embryo selection, voluntary interruption of pregnancy), as the development of research on gametes and embryos could in principle open up interesting alternatives to these practice. These aspects will not be specifically addressed in this opinion.

4. NBC reflections

4.1 Animal testing

The community of scientists and bioethicists agrees on the need to further laboratory experiments on non-human subjects in order to refine the *gene editing* technique on germ cells and embryos, starting with animal models, and controlling the effects on a reasonable number of generations.

[21] In this case, it refers to non-in vitro embryos until the 8th week.

[22] On enhancement see the NBC opinions**,** *Neuroscience and pharmacological cognitive enhancement: bioethical aspects*, 2013.

This type of research raises the issue of the ethical limits of genetic testing to which animals would be subjected to: a problem common to all animal experimentation, but which, in this specific case, is even more accentuated given the possibility of introducing, with relative ease, genetic modifications of much greater importance compared to those carried out so far. The Committee considers the testing of *gene editing* on animals to be ethically legitimate, recalling established bioethical principles and prevailing international and national legislation[23].

4.2 *Gene editing* on human somatic cells

The Committee feels that it is ethically legitimate and desirable to develop gene therapy on somatic cells, that is to say, cell correction/replacement on a part of the body in a controlled way, in compliance with the customary criteria of experimental practices for therapeutic purposes: risk/benefit proportionality, informed consent and justice.

The Committee is aware that these are high risk therapies and believes that, in the absence of therapeutic alternatives, it is important to offer this opportunity to severely ill patients. However, this field represents important promise (both in basic research and clinical applications) in terms of "precision medicine" on somatic cells and it would be a loss for scientific progress to suspend experimentation.

The objections to the use of *gene editing* applied to somatic cells, especially those based on initial protocols, are: the possibility of developing secondary immune reactions to the introduction of the vector (virus), which is used to carry the gene into the cell that is genetically foreign to the host; the possibility that the modified vectors not only reach the target cells of the therapy, but also other cells and tissues, possibly colonizing the germ cells[24], the possibility that the vector regains infectiveness and becomes a pathogen to the host; the possibility that the "new" gene may be inserted into a wrong portion of the genome and induce unpredictable pathological forms. Today, on the whole, these risks are relatively remote, since the experiences gained in the early pioneering years of genetic therapy have enabled us to develop reliable and secure protocols.

Despite these risks, the Committee believes that there is a moral obligation to cure present generations, using all the means available to science and technology. In this case, any damage, indirect consequences of acceptable and non-transmissible therapy, should be considered differently from the programmed modification of germ cells and human embryos. Similar to the situation with regard to possible serious side effects of potentially effective therapies, and in the case of otherwise untreatable pathologies, this risk is believed to be acceptable in view of the therapeutic potential of *gene editing* (and any other gene therapy) and therefore does not involve the ban on application on somatic cells.

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[23] On this topic, see NBC, *Alternative Methods, Ethics Committees and Conscientious Objection to Animal Testing*, 2009; *Bioethics and Veterinary Science, Animal Well-being and Human Health*, 2001; *Animal Testing and Health of Lliving Beings*, 1997.

[24] M. Kaplan, I. Roy, *Accidental Germ-line Modification Through Somatic Cell Gene Therapy*, in “American Journal of Bioethics”, 2002, vol. 2, n. 1. This possibility has been taken into account in the nineties, so much so that in the Explanatory Memorandum to the Oviedo Convention, paragraph 91, care was taken to point out that the prohibition in art. 13 for genetic modification, does not extend to somatic interventions that "could have undesirable side effects on germ cells".

Recently however, the clear distinction between the ethical issues concerning the modification of the germline and those of the somatic line has been called into question by some, since at least some critical issues overlap. This is because of the difficulty of circumscribing the applications of *gene-editing* to therapeutic uses, given the increasing difficulty in distinguishing between therapeutic uses and non-therapeutic (*enhancement*) uses in the scenario of development of medicine that is increasingly oriented towards prevention; this is so for the possible (albeit rare phenomenon) modification of germ cells in the *gene editing* of somatic cells whose "undesirable effects", would nevertheless result in a modification of the genetic heritage of the descendants.

5. Application of *gene editing* on human gametes for in vitro conception of embryos and on human embryos

As evidenced by the international debate, there is a point of convergence in not proceeding, at this stage, to the clinical use of gene-editing on gametes to be used for fertilization and human embryos to be implanted; while opinions are divided on whether or not in vitro research should continue on either.

The Committee agrees on the illicitness of the transfer into the uterus of modified embryos due to the high risks for the unborn child at the present state of scientific research. This involves, particularly the risk of not correcting the genetic defect and/or introducing unwanted modifications that can induce and transmit serious diseases. The NBC therefore agrees with the opportunity of a moratorium on the research leading to the transfer of a modified embryo into the uterus, the onset of pregnancy and birth. Research often referred to in international documents as "clinical research" (meaning the phase of *clinical trials* on humans), clearly distinguishing it from basic research, as while clinical research, understood in the broadest sense, is a process, it is however possible to distinguish between the various aspects[25] and the different purposes: among these the final application stage, namely that of "clinical research" in the strict sense. Some members of the Committee, who do not want to move away from the terminology of the aforementioned documents, restrict use of the locution because they consider that the "basic" research stage should not be confused with that of clinical application; in their opinion, the latter would also include research on embryos modified using gene editing and implanted in the uterus to give rise to pregnancy and birth, which, as such, must however comply with the stringent regulatory criteria of clinical *trials*, in order to be undertaken: firstly, the favourable benefit/risk ratio. Other members prefer to define this type of research as "in vivo" research, as they believe that the classifications "basic research" and "clinical research" do not necessarily indicate rigidly separate

[25] Cf. European Science Foundation, *Implementation of Medical Research in Clinical Practice*, 2011, Executive Summary, Foreword Look: “Clinical research can be looked upon as a broad term that includes basic-oriented research, disease-oriented research with animal models, i.e. translational research, patient-oriented research and outcome research. The terminology is varied across Europe and the rest of the world, but in spite of this it is important to stress that all aspects of biomedical research are necessary. Basic oriented research aims to generate knowledge but may perhaps not be immediately relevant for practical applications in patient care. Clinical research is described by others only as *research protocols involving patients*. For everyone involved in this research area the important thing is that the whole spectrum of research is essential, from basic, through translational to patient-oriented research and back again. One part is ineffective without the other”, p. 5, www.esf.org/fileadmin/Public_documents/Publications/Implem_MedReseach_ClinPractice.pdf.

compartments[26]. Indeed, the term "basic research" would presuppose research for an exclusively cognitive purpose (whose path, however, has no pre-established stages and is inherently unpredictable) and may refer both to the studies on embryos and gametes in the laboratory (in vitro) that on embryos in the uterus (in vivo). In this sense, it is preferable to avoid attempts to categorize research on the basis of purposes; instead, reference should be made to two distinctly separate areas, "in vivo" and "in vitro".

In addition to the different terminology options, the Committee expresses differing arguments and positions on the legitimacy of in vitro (or basic) research of *gene editing* on gametes and human embryos which, looking ahead could allow the correction of disease genes before conception or implantation, if the conditions for effectiveness and safety of the technique were set at a level of international consensus and it passed to clinical use.

5.1 The viewpoint of those in favour of basic research on *gene editing* of gametes not intended for conception and on non-implantable embryos[27]

Those who support the pursuit of basic research on gene editing, even in the preventive perspective of eliminating genetic mutations at the basis of serious diseases before the onset of pregnancy, consider it reasonable and ethically legitimate to experiment on residual embryos derived from "in vitro" fertilization techniques which for biological reasons or because permanently in a state of abandonment, are unable to be implanted[28].

Firstly they note that blocking basic research on gene editing, in addition to the moratorium on "clinical research", would result in a total ban on *germline* research. This would undermine the meaning and nature of the moratorium itself, which, while aiming to protect human health from experiments which involve an unacceptable risk, does not preclude direct research to perfect the technology and study the genetic and molecular mechanisms that originate each individual life[29]. Moreover, transparent basic research on in vitro gametes

[26] It is noted that biomedical research can be subdivided into typologies classified with various conventional denominations. The term "basic research", generally opposed to "clinical" presupposes, research for purely cognitive purpose, and can refer to both to the studies of gametes and embryos in the laboratory (in vitro) and to embryos in the uterus (in vivo). Several international documents also refer to a third type of research, the so-called "preclinical" research, for which it is difficult to identify a unique definition, both in terms of its purpose and object, being able to relate to the experimentation both in laboratory and on the human body. As the European Science Foundation points out, the terminology "varies between European States and the rest of the world" on the basis of duration (European Science Foundation, *Implementation of Medical Research in Clinical Practice*, 2011, www.esf.org /fileadmin/Public_documents/Publications/Implem_MedReseach_ClinPractice.pdf.
The distinctions "in vitro" and "in vivo" sometimes correspond, respectively, to "basic research" and "clinical research", but often this is not the case, as evidenced by the ESF cited above, and in the "clinical research" certain types of "research with biological materials of human origin" are included.

[27] Profs. Battaglia, Canestrari, Casonato, de Curtis, Di Segni, Flamigni, Garattini, Toraldo di Francia, Zuffa.

[28] NBC, *The Destiny of Embryos Resulting from Medically Assisted Procreation (MAP) and not Complying with the Conditions for Implantation*, 2007: some members of the NBC had already expressed their views in the opinion regarding the lawfulness of experimentation on non-implantable embryos affected by serious anomalies.

[29] Cf. Perspectives: *Kathy Niakan: at the Forefront of Gene Editing in Embryos,* www.thelancet.com, Vol. 387, March 5, 2016, p. 935.

and embryos should be encouraged precisely in order to decide in the future, if, and which, clinical applications may be considered scientifically possible and ethically licit[30].

Recalling the invitation of the December 2015 International Summit, mentioned above, it can be said that the development of basic research is needed precisely "to understand the biology of human embryos and germ cells." This research should use human embryos at an early state, as previously indicated, and germ cells, with the guarantee that they are not implanted to start a pregnancy. An example of informed research on these criteria is represented by the experimentation authorized by the Human Fertilisation and Embryology Authority (HFEA) in February 2016 and started at the Francis Crick Institute in London. Basic research aimed at acquiring knowledge on the early stages of embryonic development (up to the eighth day after fertilization) in order to search for some of the causes of early abortion and infertility[31]. A first important reason to continue research using embryos that would be destroyed anyway therefore lies in the increase of knowledge acquired through the CRISPR-Cas9 technique[32].

There are remarkable scientific opinions about the importance of basic germline research, both for cognitive purposes and for potential therapeutic applications. So the Harvard Medical School geneticist George Church has taken a stand against those who argue that there is no need to continue research on gametes as existing technologies can be used to select embryos. Church notes that, given the growing number of cases in which several genes are involved in a disease, many embryos may be destined not to be implanted and then destroyed. In this perspective, *editing* would considerably increase the likelihood of having healthy embryos[33] Nevertheless, research aimed at the selection of gametes should also be promoted in parallel.

Even, the Nobel laureate Craig Mello, a geneticist at the University of Massachusetts in Worcester, while imagining a future, still distant, in which the modified germline can protect men from many serious diseases, believes that in the short term there may be "*good reason to experiment with discarded embryos or embryonic stem cells for research purposes*." George Daley, a biologist at Harvard Medical School declared himself in favour of *editing* human embryos in vitro, this technique could find an answer to many scientific questions which, although they have nothing to do with clinical application, are crucial for understanding the early stages of human development.

Taking the same line, the National Academy of Science and the National Academy of Medicine conclude the chapter dedicated to *Basic Research Using*

[30] See. One of the recommendations contained in the letter of "Science" already mentioned (*A prudent path forward for genomic engineering and germline gene modification,* March 19, 2015).

[31] See Perspectives: *Kathy Niakan: at the Forefront of Gene Editing in Embryos*, cit., P. 935. Niakan is optimistic about the possible clinical implications in the short term in at least two areas: early abortion and female infertility.

[32] Cf. D. Cyranoskin, S. Reardon, *Embryo Editing Sparks Epic Debate,* in "Nature", 29 April 2015, *Applying gene editing to human embryos could answer.*

[33] Cfr. David Cyranoski (2015), *Ethics of Embryo Editing Divides Scientists*, in "Nature", 519, 272 (19 March 2015) doi: 10.1038/519272a. This does not mean that research aimed at a more accurate selection of gametes should also be promoted in parallel, which may be useful in many cases, but not in all.

Genome Editing of the aforementioned document *Human Genome Editing: Science, Ethics, and Governance* of January 2017[34]

Choosing to go ahead with basic research, even if at the moment it is not known whether the safety and efficacy criteria necessary to move to the clinical trial stage will ever be achieved, is also motivated by some potential benefits of genetic intervention on the germ line compared with those on the somatic line: while somatic gene therapy does not eliminate the genetic defect at the root and hence the individuals undergoing this therapy are destined to transmit it to the offspring, gene therapy on the germline would potentially be able to eradicate in a preventive and definitive way mutations at the basis of serious diseases in the interest of future generations.

Gene editing on the germline would fit so positively into the prospect of medicine increasingly oriented towards prevention as regards treatment. It is also true that the current development of prevention towards the promotion of "healthy" lifestyles (which are increasingly part of the "normal" life of "healthy" people) tends to blur the differences between what is protection from diseases in the strict sense and what is "enhancement" of appreciable human characteristics. It is precisely this peculiarity which is likely to interfere with the concerns raised by the public in the prospect of modifying the genetic heritage of future generations, increasing fears. The ghost of science that wants to reshape the "human species" can, however, be exorcised on the one hand through providing correct information and proper instruction of the public ethical debate[35]; and on the other, by pushing the debate itself beyond the issue of safety parameters and effectiveness of technologies, to begin envisaging appropriate public policies should the parameters mentioned above be met and research therefore move to the application phase: identifying, for example, the authorities responsible for deciding on the modalities and limits of application of CRISPR-Cas9; giving priority to serious illness and in particular to the correction of genetic mutations known to be the cause of serious life-threatening diseases for which there are no effective treatments. That being said, the members of the Committee who can identify with this position point out that, a general prohibition on the use of supernumerary embryos ends up restricting other constitutionally-founded rights and interests. In this perspective, it is deemed necessary to seek a balance between the different requirements of protection[36]. Consequently, it seems constitutionally reasonable and correct from a bioethical point of view, to foresee the use of embryos which can no longer be used for reproductive purposes - under certain conditions including the prior consent of

[34] In the text (p. 60) states: «*Important scientific and clinical issues relevant to human fertility and reproduction require continued laboratory research on human gametes and their progenitors, human embryos and pluripotent stem cells. This research is necessary for medical and scientific purposes that are not directed at heritable genome editing, though it will also provide valuable information and techniques that could be applied if heritable genome editing were to be attempted in the future*».

[35] For example, writes in the Blog "Of Science, CRISPR-Cas9 and Asilomar", 4 April 2015: "*The non medical demand is the real fear of most people. But it turns out that, after hundreds of billions of dollars spent, we know surprisingly little about the genetics of disease. We know almost nothing about the genetics of 'enhancement'*".

[36] On the subject cf. Demetrio Neri (2015), cit., pages. 216-217, proposes to entrust to the WHO the drawing up of a list of diseases nominated for initial germline gene therapy protocols. Among the diseases that the author could include in the first list are beta-thalassemia, cystic fibrosis, Huntington's disease. See Luigi Naldini's response in "Nature Biotechnology", 2015, vol. 33, n. 5, May 2015.

those who created them - to ensure the interest of scientific research aimed at the protection of (individual and collective) health.

5.2 The viewpoint of those expressing doubts about the reasonableness and appropriateness of in vitro experiments involving *gene editing* of gametes intended for reproduction and human embryos not intended for implantation[37]

Applied to human beings, *gene editing*, particularly in the CRISPR-Cas9 variant, is a technique discussed in this opinion because it aims at verifying the possibility of preventing at least in the future, through "cure", certain genetically determined diseases which may be carried in gametes or the embryo.

In this context, therefore, the central bioethical problem remains, not only as for any experimental technology, the guarantee of conditions of safety to pass from experimentation on animals to experimentation on humans, but also experimentation on embryos for curative purposes, if the CRISPR-Cas9 technique can achieve at least in future predetermined expectations and justify useful and reasonable experimentation. This does not mean a discussion on whether it is ethically licit to generally experiment on residual or abandoned embryos derived from in vitro fertilization techniques which, for the most diverse reasons, can not be brought to birth, and therefore if such research may be beneficial for other purposes in addition to curing the embryo (such as understanding the early stages of embryonic development, researching some of the causes of early abortion and infertility, protecting humans from serious illnesses through modified germ line, etc.). The scientific doubts and therefore moral doubts put forward by some members of the Committee are therefore limited to a reflection on the use of *gene-editing* on gametes and human embryos for the above-supposed purpose: to cure and bring to birth embryos originally affected by certain genetically determined diseases.

(a) Regarding human gametes: doubts about experimentation on gametes to be implanted, the licitness of experiments on gametes not intended for implantation

The CRISPR-Cas9 technique, with the scientific purpose of the gametes to correct their genetic defects before they are used for fertilization, may be justified by the presence of transmissible mutations in the zygote. The *gene editing* applied to germ cells would therefore be intended to modify/repair an aploid genome, carrying a mutation uniquely attributable to a known pathology. Consequently, the modification introduced in the gamete would be inherited from the zygote.

But what indication is conceivable for a modification of this type? Certainly not the *de novo* genetic mutations of gametes, that is, those that arise spontaneously, favoured by the mechanism of gametogenesis and environmental factors, which in males show a clear correlation with age (it is estimated that a 30 year old man transmits on average about fifty new mutations and these become about 90 at about the age of 40). It is a case of possibly correcting the mutations transmitted by the producer of the gametes. In fact,

[37] Profs. Amato, Caltagirone, Dallapiccola, D'Agostino, Gensabella, Morresi, Palazzani, Proietti, Scaraffia.

since the human species is diploid, by definition, whoever is affected by a dominant autosomal disease, if heterozygous (that is, having only one mutated allele) has, in addition to the mutated allele, a normal allele, and therefore 50% of its gametes, the normal ones, could be selected in the future for in vitro conception, rather than imagining using *gene editing*, always in the future, to modify the gamete that carries the hereditary mutation.

The healthy (heterozygous) carriers of a recessive autosomal mutation responsible for homozygous disease are in the same situation, that is, they produce half the gametes without mutation, while people with autosomal recessive condition have both alleles mutated (homozygotes). The only case in which the offspring would always be affected is that of "marriage between two affected homozygotes"[38] (subjects possessing both alleles of a mutated *locus* and therefore produce only mutated gametes) from the same disease. However, this would be extremely rare. In all other situations, by selecting the gametes, it would always be possible to prevent that the conceived suffers from the disease[39].

In conclusion, in the case of simple diseases (mendelian), it is not easy to imagine what indications (except for exceptional situations) could justify the use of genetic modification of gametes to correct a disease risk related to the presence of a segregating mutation. At this time it would be more reasonable to invest in research aimed at selecting gametes rather than subjecting them to *gene editing*. In this regard, it should be remembered that as far as the feasibility of *gene editing* on gametes is concerned, it would not be "just" to develop a technique capable of correcting potentially transmissible mutations without inducing secondary errors, but also of modifying the aploid genome without interfering with the *imprinting* process, which is critical for the proper functioning of the gamete. For cases - bioethically relevant but numerically residual - in which the selection of gametes could not be used to prevent the conception of a person suffering from a hereditary disease (e.g. marriage between two homozygotes affected by the same disease), the following arguments apply regarding *gene editing* on human embryos and the problem of the "response threshold".

Therefore, in the light of the analysis of possible cases and explicit scientific arguments, some members of the Committee believe that *gene editing* on human gametes used for reproductive purposes is not scientifically and ethically justifiable for the high risks that it may currently have (in terms of the introduction of new mutations on embryos produced by gametes) and non-utility for selective purposes, which can be achieved in a less risky way (by orienting research towards the selection of gametes intended for the production of human embryos).

[38] In genetics, the term marriage is synonymous with *mating*, without the meanings of current language.

[39] The marriage between a person suffering from a recessive autosomal disorder and a person with a wild genotype (a gene that expresses the natural phenotype, that is, not mutated, for a particular character, the wild phenotype is the most frequent in a natural population) would produce only zygotes that are "healthy obligatory carriers" and therefore not affected. In the case of marriage between a homozygous affected and a healthy carrier of the same disease gene, 50% of those conceived would be unaffected and in theory could only produce zygotes without the mutation, after gametic selection. In the case of X-linked recessive diseases, 50% of the gametes produced by a female carrier are, however, normal.

Notwithstanding the non-justifiability of the modification of gametes in the terms and for the purposes illustrated above, in vitro research on gametes for non-reproductive purposes remains licit.

(b) Regarding research on human embryos not intended for implantation.

As for experimentation of *gene editing* on human embryos, there is the current ethically problematic nature of the technique. To state this is not to call into question the ethical principle of the possible objectives of research namely prevention and possible cure for serious genetic diseases; if anything, the problem lies in the lack of technical safety and the inability to assess its effectiveness, in addition to matters of justice concerning the distribution of scarce resources.

The reasons are as follows:

- *Lack of safety*

While recognising the importance of even experimental research on the embryo for preventive and therapeutic purposes for the health of the same embryo (in the absence of therapeutic alternatives and with informed consent) today reasons of caution lead us to believe that using the CRISP-Cas9 technique is unjustifiable due to its high risk and unpredictability. There could at least be a minimal ethical criterion for applicability introduced: the technique could be considered reasonable and licit when it is at least demonstrated that the genetic modification intervention on diseased human embryos induces less damage than that caused by the disease which it is intended to correct. This criterion presupposes as a condition the possibility of scientifically and reasonably estimating the probability and type of possible adverse events and the side effects of the modification intervention both on the embryo subjected to experimentation on the embryo for therapeutic purposes, and on subsequent generations. This estimate is, at the present time, impossible.

The progress of medicine has also been achieved thanks to pioneers whose interventions were at the very limits of safety, in terms of possible risks for patients, this being carried out on consenting adults (an example for all, is organ transplants). If it is justifiable in the context of somatic *gene editing* (on adults, on born children and even on fetuses) to face even high risks in conditions involving serious incurable disease destined for certain death in a short time, without therapeutic alternatives, with suffering and serious disabilities, in the case of embryo *gene editing*, the risks and possible unforeseeable damage involve a human subject at the early stages of development at a totipotency stage (without localization of modifications to an organ or a specific function). So, what is desired is the correction of a diagnosed disease, with the high risk of introducing other potentially inheritable mutations (and therefore other pathologies) that may have even more serious consequences than the very pathology to be corrected.

- *The inability to evaluate effectiveness*

While it is possible to monitor the success of correct modification, it is problematic not only to identify any possible induced mutations but also to ascertain their overall effects on the functional aspects of the embryo. To be sure of the results of *gene editing*, it is not enough to stop at the applying of "molecular scissors" to the embryonic genome – that is to say, research limited

to laboratory studies is not enough - but, as with all other applications of *gene editing*, it is necessary to verify the functional effects on the modified genome, also taking into account any *off-target* effects, that is, any unwanted modifications.

It would be necessary to implant the embryo into the uterus, to monitor embryo-fetal development, and to check the condition of the modified baby, to follow its development and to carry out a transgenerational *follow-up*. Therefore, *in vivo* research[40] would be necessary.

In vitro research on *gene editing* in embryos, in its present state, necessarily implies research using the uterus without which it is not possible to know if modifications introduced in the edited genome are really "curative" and therefore effective and not harmful. As clearly stated in the aforementioned letter published in "Nature": "*the precise effect of genetic modification to an embryo may be impossible to know until after birth. Even then, potential problems may not surface for years*". It is evident that the short period in which the human embryo can be examined in vitro is not sufficient to verify its complete development and therefore to ascertain the outcome of *gene editing*, which can be effectively monitored by exploring *in vitro* somatic cells and, subsequently, *in vivo* in the various applications, as widely documented in scientific literature[41].

The only possibility would be to accept the birth of genetically modified embryos potentially at risk of carrying undesired mutations induced by the technique; a risk that, in theory, in time could be reduced. This would mean newborn babies programmed, modified and specially created to improve a technique. This is an unacceptable proposal given that right from the start of clinical application this risk should be almost zero.

The birth of subjects with possible "defects" linked to the current unsafe technique makes it ethically problematic at this moment. Similarly to the discussion on cloning (referring in this case to "reproductive" cloning), it is difficult to imagine the licitness of research that, in order to acquire scientific certainty, should have to proceed gradually by experimentation on human beings without guarantees for their health. As with cloning, even for this technique, the planned and conscious birth of an unpredictably "defective" human being is unacceptable ethically and juridically.

Gene editing experiments on embryos can only be taken into account when scientific knowledge allows them to identify the "response threshold" by other methods, i.e. without the need to start a pregnancy and to carry out the generational *follow-up* on the newborn child. Serious perplexities regarding the

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[40] "*The precise effects of genetic modification to an embryo may be impossible to know until after birth*", in "Nature" (nota 2); see, as an example, Greco E., Minasi M.G., Fiorentino F., *Healthy Babies after Intrauterine Transfer of Mosaic Anauploid Blastocysts*, N Engl J Med., 2015 Nov 19; 373 (21): 2089-90, doi: 10.1056/NEJMc1500421, www.nejm.org/doi/full/10.1056/NEJMc1500421; resumed www.adnkronos.com/salute/medicina/2015/11/20/nai-italia-primi-bimbi-sani-embrioni-malati_sQj3LAvGIOZTKZrsfYKqnM.html.

[41] In this respect, it can be understood why, at the time of the approval of this opinion, compared with the 3,900 scientific articles concerning CRIPR-Cas9 (see Nuffield Council), only two studies have been published on the *gene-editing* of human embryos, one of which was refused by the journals *Science* and *Nature* which perhaps would not have missed out on the opportunity of the exclusive publication of the first *gene editing* of human embryos if it had been considered significant from a scientific point of view). The technique has enormous potential, but for human application at this time it is only possible to completely verify its effectiveness in somatic cells.

application of *gene editing* on the embryo to prevent hereditary diseases also emerged from the document *Human Genome Editing: Science, Ethics and Governance* in January 2017 by the National Academy of Science and the National Academy of Medicine[42]

Therefore, the currently practicable route for clinical application of *gene editing* is that of somatic gene therapy, that is, on subjects that have already been born.

In principle, it is reasonable to expect any errors or adverse events to be more controllable compared to intervention on the embryo that is intrinsically irreversible and potentially transmissible to subsequent generations, although the consequences linked to the problem of being an *error-prone* technique can not be excluded even for somatic gene therapy on individuals.

The research on embryos *in vitro* without the hypothesis of implantation in the uterus (i.e. without the possibility of verifying its effectiveness) is therefore an end to itself and therefore not useful. And if every research and experimentation has to be legitimized by a "scientific rationale," this is even more true in the field of embryo research, the legitimacy of which is justified by the prospect of producing greater scientific progress and the lack of alternative methods of comparable effectiveness.

- *The question of justice*

Within the framework of the criterion of justice in the distribution of scarce resources, it is ethically problematic to invest in research applicable to a small number of cases on not yet existent individuals, compared to research on therapy for individuals who are already severely ill.

6. Recommendations

The Committee draws attention to the importance of a wide-ranging public dialogue on the issue of gene editing and its development as induced by CRISP-Cas9 technology in the various scientific, ethical and social spheres involved. Dialogue can only begin with full information about the potential of knowledge and future applications in multiple fields, from the environment to the medical clinic, to human reproduction; about the specific ethical issues and the social consequences of the various applications; about public policies, especially the legal regulations, to assess whether these offer or not a valid framework to support and regulate the development of research.

The NBC reaffirms the importance of scientific research for both cognitive and therapeutic purposes, but also intends to take a prudent line in relation to experimental techniques which, given the current state of knowledge, have strong margins of uncertainty.

With regard to using the new technique of *gene editing* CRISPR-Cas9 in the human sphere, the subject of this opinion, the Committee makes the following observations and recommendations:

[42] The dedicated chapter (5), explains, among other things, how embryonic mosaicism is a serious impediment to the application of gene editing in the embryo; according to the authors, the potentially promising alternative could be to edit the precursor cells of gametes: "*The future prospect for heritable germline genome editing in humans will change dramatically if genome editing in progenitors of human egg and sperm becomes a reality*".

1. It recommends that public debate should not only take into account the issues of the effectiveness and safety of the technique, but also, as regards *gene editing* on the germline, the ethical implications of the introduction of potentially transmissible genetic modifications to future generations. This implies the need to find internationally shared rules to govern biomedical research and to identify the areas of application on which to find widespread consensus.

2. It proposes to continue *in vitro* and animal experimentation, in accordance with the internationally shared ethical rules of such experimentation, in order to test the safety and efficacy of these technologies.

3. It considers ethically acceptable and desirable a strong promotion of human somatic cell research both in laboratory research and in clinical or *in-vivo* research. With the recommendation that the ethical criteria of any highly innovative research should be followed.

4. Compared with *gene-editing* on the human germline, given the current state of scientific knowledge, it considers ethically unjustifiable experimentation on gametes intended for conception and human embryos destined for implantation in order to obtain a pregnancy and agrees therefore with most of the scientific community on the advisability of a moratorium on clinical research or *in-vivo* research until the necessary conditions of safety and efficacy of the technique have been met.

5. As for experimentation of *gene editing* in the laboratory on gametes not intended for reproduction and on *in vitro* embryos not intended for implantation:

5.1 Some members believe that the moratorium on clinical research should not extend to basic *in vitro* research of *gene editing* on gametes not intended for conception and on human embryos not intended for implantation. They therefore hope that *in-vitro* basic research will proceed not only to improve the technique but also so as not to block *in toto* research on *gene editing*, preventing any future transition to clinical application, but also in order to increase knowledge of the processes of cell development, related diseases and human health in general;

5.2 For other members**,** if the purpose of CRISP-Cas9 technology is to achieve the possibility of genetically modifying gametes and embryos for therapeutic purposes, for the birth of these embryos, they believe that the scientific community should specify valid ethically acceptable criteria for safety and effectiveness before moving to *in vivo* experimentation of *gene editing* in human beings. The effectiveness of *gene editing* is the focus of scientific experimentation on many cellular, plant, animal, and human systems (on somatic cells) that could in future open new possibilities or hypothesize new techniques that can be safely applied to human embryos for therapeutic purposes. Given that the current state of *gene editing* on embryos *in vitro* does not allow to scientifically establish its efficacy and safety, given the impossibility of verifying the results of genetic modification carried out on embryos and gametes for fertilization, unless at birth or even later, some members of the Committee believe that such research is not currently justified. They consider legitimate *in vitro* experimentation on gametes not intended for reproduction.

APPENDIX

1. The context: a brief history of gene therapy

The birth of genetic engineering can be traced back to the discovery of recombinant DNA and restriction endonucleases, an enzyme used by bacterial cells to fragment viral DNA. This discovery dates back to the 1960's and in 1978 it motivated the award of the Nobel Prize to Hamilton Smith, Dan Nathans and Werner Arber. Later, it was put to good use in terms of application by Paul Berg, Herbert Boyer and Stanley Cohen, who in 1982 obtained insulin synthesis using genetically modified bacteria. When scientists understood the potential of this technology, both in basic research and in possible applications, they stopped, more or less spontaneously, with a moratorium culminating in the Asilomar conference. In 1972, the idea arose to apply this methodology in the development of gene therapy protocols.

After an unfortunate false start in 1980, involving an Italian scientist and an Italian patient, the first gene therapy protocols[43] were developed at the end of the 1990s, starting from the one approved by the FDA on 14 September 1990 and immediately experimented on a child with adenosine-deaminase deficiency (ADA). The partial success obtained encouraged the application of somatic gene therapy to other diseases[44]. One of the goals of the researchers of those years was not so much that of replacing the mutated gene, but to fix it *in situ*, to interfere as little as possible with the physiological function of the cell. Significant progress in the precision of insertion of the gene was obtained from research by Mario Capecchi, Nobel Prize in 2007, who developed homologous recombination techniques. Research has then proceeded in the direction of combining the precision of *gene targeting* with a higher yield in terms of modified cells.

2. International Documents on gene therapy in the 70's/90's

Reference should be made to the Asilomar conference of 1975, which was convened and chaired by Paul Berg, who proposed a moratorium on the method of inserting a recombinant DNA formed by the SV40 virus (which was known to be carcinogenic) and a bacteriophage, in an Escherichia coli cell. The moratorium was agreed on in defense of biosecurity, for health and environmental risks.

In particular, with regard to genetic modification of the germ line, a similar position had already been expressed by the scientific community from the Final Declaration of the CIOMS Conference (*Council for International Organizations of Medical Sciences*) held in Inuyama and Tokyo in 1990, in which any clinical application of germline gene therapy should have been bound to a number of conditions that the research of those years was unable to guarantee. The

[43] In particular we recall Strimvelis to treat patients with severe combined immune deficiency disease associated with adenosine deaminase deficiency, the so-called " bubble baby", forced to be isolated from the world (in a "bubble"), and is the result of Italian research. Currently, other pathologies, even less rare, such as beta thalassemia are being studied.

[44] Some patients have died from gene therapy trials. Jesse Gelsinger, an eighteen-year-old boy with ornithine decarboxylase deficiency (which causes liver metabolic failure) died on September 17, 1999, four days after being treated with the correct gene using a modified adenovirus vector. This first case has raised scientific and ethical questions worldwide. In 2003, a child with SCID-X1 (X-linked severe immunodeficiency) was treated with an experimental gene therapy and died.

position of "moratorium" remains the most widespread not only in the scientific community but also within the bioethical community.

It is the line of the *Group of Advisers on the Ethical Implantation of Biotechnology to the European Commission* in the opinion *The ethical implications of Gene Therapy* (Brussels, 1994), which declared germline gene therapy to be ethically unacceptable, stating “for the time being due to complex technical and scientific problems that remain unsolved”.

The NBC, in 1991, in its opinion on Gene therapy wrote: "Gene therapy directed to germ cells is, at present, ethically and scientifically impracticable [...] the impropriety of the intervention comes from the fact that the conceptual and technical bases for predicting the effects of germinal gene therapy on the development of the individual and the individual’s offspring do not currently exist; this should not however preclude that in future it may be possible, with the acquisition of new knowledge and the development of more efficient techniques, to achieve targeted integration of genes on the germ line without altering the structure and function of the genome." The moral illicitness of genetic manipulations of germ cells and embryos was not established for reasons of "principle" but reasons “of fact” linked to the risks currently (at the time) related to the technique and damage to offspring. The Committee therefore felt that, insofar as research had been able to solve technical problems of safety (the new techniques promise precisely this), the judgment of illicitness could have been revised, not depending only on reasons of principle.

More restrictive is the position adopted by art. 13 of the Council of Europe Convention on Human Rights and Biomedicine (Oviedo Convention) (1997): "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".

3. Some international papers and interventions on *gene editing*

Important international and European bioethics organizations have also recently intervened on the issue, their recommendations are, however, not always consistent.

The UNESCO International Bioethics Committee in the *Report of the IBC on updating its reflection on the human genome and human rights* (2 October 2015) focused on the ethical challenges of the new technology, recalling the value of the human genome as the "heritage of humanity" and highlighting the indispensable need for security and consensus. Conscious that the destruction of embryos implicated in some of these technologies has brought to the fore the well-known ethical controversy on the interpretation of the principle of respect for human life and, consequently, the statute of the zygote, embryo and fetus, the document highlights the impossibility of reaching a shared position on this matter. The report also considers this technique to be problematic even from the point of view of the principles of equal dignity of human beings and of justice that is, of sharing the possible benefits of research, as well as invoking art. 13 of the Oviedo Convention, which prohibits the modification of the hereditary genome.

The European Group on Ethics in Science and New Technology has approved a *Statement on Gene Editing* (January 2016) in which, having recognized the enormous development of gene therapy techniques over the past 40 years and drawn a distinction between germline and somatic therapy, it believes that the issue requires "careful consideration, given the profound

potential consequences of research for humanity" and adheres to the moratorium on *genome editing* for reproductive purposes of embryos and gametes, considering further technological development to be indispensible before moving to clinical trials. In this context, it introduces a distinction between the research that produces clinical applications and basic research, also recognizing the difficulty of demarcating the border between the clinical applications for therapeutic purposes and those for the purposes of enhancement. The EGE also hopes that the debate will be as inclusive as possible in terms of different perspectives and competences and will not be limited only to security and potential health risks, but also address issues of dignity, justice, equity, proportionality and autonomy; extending also to non-human applications, in particular to the consequences on the biosphere and the environment. However, the Committee is divided on its ethical position: some members believe that modification of the germline for reproductive purposes to be ethically unjustified, asking for a moratorium even on basic research until a regulatory framework adapted to the new possibilities has been developed, given, in their opinion, the fine dividing line that separates it from applied research. However, other members believe basic research to be justified.

The Council of Europe's Bioethics Committee (DH-BIO) issued a *Statement on genome-editing technologies* on November 13, 2015, recognizing the potential of this research. In the Statement, the extensors referred to art. 13 of the Oviedo Convention, considering that it provides the "benchmarks of the international debate" on the fundamental problems raised by new technologies and recalls that this debate was envisaged in the same Convention in Art. 28.

In the International Society for Stem Cell Research (May 2016) *Guidelines for Stem Cell Science and Clinical Translation*, one of the major novelties compared to the previous guidelines (*Guidelines for the Conduct of Human Embryonic Stem Cell Research*, 2006; *Guidelines for the Clinical Translation Of Stem Cells*, 2008) specifically concerned *gene editing* techniques. The new guidelines state that *gene editing* techniques, as well as other genomic modification techniques that permanently alter the germ line should be prohibited for reproductive purposes, at least at this present moment. For any possible intervention regarding heritable *genome editing*, the guidelines indicate strong safety evidence and broad public consensus[45] as being essential.

In September 2016, the Nuffield Council on Bioethics published *Genome editing: an ethical review*, a document aimed at "identifying and defining the ethical issues raised by recent advances in biological and medical research in order to anticipate and respond to possible social concerns". This is a first document to be followed by a second text aimed at deepening the regulatory profiles of using the most innovative techniques[46].

[45] Recently, the US National Academies of Sciences, Engineering and Medicine have published the *Gene Drives on the Horizon* report, which has generated a very lively debate in specialized literature and media. Although the report relates to non-human organisms, it points out that genetically engineered organisms "are not ready to be released into the environment", as it is still necessary to progress in research (Committee on Gene Drive Research in Non-Human Organisms: Recommendations for Responsible Conduct On Academic Press, 2016), on Life Sciences, Division on Earth and Life Studies, National Academies of Science, Engineering and Medicine, *Gene Drives on the Horizon*: *Advancing Science, Navigating Uncertainty, and Aligning Research with Public Value*. Washington, D.C., National Academies Press, 2016).

[46] http://nuffieldbioethics.org/project/genome-editing.

4. Italian Regulation

Article 13 of Law 40/2004 prohibits any experimentation on each human embryo (paragraph 1) and allows clinical and experimental research on each human embryo (paragraph 2) "when it is finalized to the exclusive therapeutic and diagnostic protection of the health and development of the embryo concerned and when no alternative methodology is available".

The Industrial Property Code[47] is framed in a different context which, in compliance with Directive 98/44/EC on the legal protection of biotechnological inventions, reiterates, in Art. Article 81d (1) (b) (2), the prohibition on patenting the «modification processes of the germinal genetic identity of the human being». The impossibility of patenting and therefore of commercial exploitation does not imply a ban even on experimentation on germinal genetic identity but highlights basic ethical concern regarding the problem of respect for human dignity and integrity[48].

The simplification of germinal genome modification techniques requires a serious reflection on these norms. On the one hand, their maintenance could be a serious obstacle to the refinement of technologies and their controlled and generalized usability, which are the most common effects of a patent. On the other hand, precisely the possibility of patenting might constitute a boost, difficult to control, to the possible commercialization of certain characterization of the human body, radically affecting some of the fundamental principles of the Oviedo Convention from art. 1 on respect for personal integrity to art. 21 on the ban on making the human body and its parts an object of profit[49].

Personal remarks

A personal remark signed by Prof. Carlo Flamigni

At a recent plenary meeting, after listening (with great interest and curiosity) and appreciating the long discussion in the Committee on the issue of *gene editing*, I made an observation on the usefulness of the scholarly debate which in my opinion was deserving of further reflection. I was obviously not convincing enough (I know that presumption is not one of the defects of the Committee) and the discussion continued unperturbed. More surprised than sorry I decided not to repeat my observation (for the fear of looking foolish) and to use the opportunity to add a few personal observations to the document in order to submit them once again to my colleagues, hoping to induce in them further reflection. I would like to make it clear that I have nothing to complain about as regards the quality of the approved document. Instead, I wonder about the appropriateness and need to consider an issue that is internal to the one we have dealt with, however at a first analysis it risks being overlooked. I will add that I will be happy if someone convinces me that my concern is meaningless and not worthy of consideration. In other words, this personal remark is not to

[47] Intellectual Property Code: Legislative Decree 30/2005 updated following the Legislative Decree of 13 August 2010, no. 131.

[48] Report from the Commission to the Council and the European Parliament on "Developments and implications of patent law in the field of biotechnology" (COM / 2005-312, 3.2.1).

[49] The Convention was ratified in Italy by law no. 145 of 28 March 2001. However, the law, although approved by Parliament, was not filed with the General Secretariat of the Council of Europe. Therefore, ratification is currently not valid. However, the Convention is largely in Italian law and is an indispensable point of reference for legislators, jurists and bioethics.

denote dissent. Rather, it is a simple additional consideration which found no place in the discussion.

This knowledgeable document which has just been approved by vote was written by a mainly “scientific” Committee which has chosen to be an academic authority and we all know that the Academy is not always interested in the usefulness of the themes on which it reflects, but it also loves reflection as an end in itself (which is still never completely useless). In more direct terms, I think the NBC discussion on *gene editing* is the least useful thing (not ugly, not bad, not wrong, just not useful) to which the Committee has devoted its plenary meetings. The reason for this judgment is that we have given two opinions, both complex and articulated (certainly and fortunately incompatible, as should almost always be the case in a descriptive bioethics document) on scientific research that has already taken off and is proceeding on its own in a country that has considered it ethically acceptable. I remind the reader that American geneticists who met in San Francisco a few months ago to reason on a research project in which they reflected on writing the human genome, to journalists asking whether they had tackled and solved the many ethical issues raised by the research, they responded: “if we don’t do it Chinese geneticists will". In the field of *gene editing*, exactly the same thing is happening, and we certainly can not accuse China of immorality, that country has already posed the question of the licitness of experimental investigations and resolved it in its own way, and it is certainly within its right to do so. Now, we do not know how this research will end, but there are only two possibilities: failure, which will make every ethical consideration useless and inform us that this path is most likely not viable; success (that is most likely, it is only a matter of time) in which case we will be faced with a problem that is as important as it is pragmatic and concrete (and this is the subject in question that I referred to in my speech), namely, how should we behave in the face of great scientific success, obtained through means that we consider to be ethically improper.

I imagine that everyone remembers the wild experiments of German physicians on the poor and desperate guinea pigs of concentration camps, experiments in which those doctors demonstrated their insane and fundamental madness (the chosen themes were mostly devoid of clinical interest and some of the research was even incomprehensible, as in the case of the experimental surgery designed to create Siamese twins). Well, let's imagine for a moment that an equally cruel but certainly more lucid madness allowed one of those experiments to be successful in a field of extraordinary clinical utility – a possible example could be breast cancer treatment - at the price of course of thousands of innocent lives, what would we do with this so complex inheritance, would we refuse it for its immorality, or should we accept it on the basis of more concrete pragmatism? Try to imagine that this choice is fundamentally decisive for the survival of the person you love most and draw your own conclusions. Clearly, I'm not making comparisons between the two researches; I only have in mind the general problem of how to behave when faced with the results obtained through investigation considered objectionable on moral grounds. Such an issue which emerged a few years ago regarding the two lines of research on stem cells that exchanged information on the new knowledge gained to the point that some Catholic bioethics declared that the potential acquisitions recorded by research considered as moral (namely, research on adult stem cells) would not be usable because it was infected by ethically unacceptable research on embryonic stem cells. I do not have a clear and

definitive position on this issue, but I ask myself questions that I struggle to answer. For example: Is it possible that the condemnations of Nazi experiments from Helsinki onwards were so numerous and spontaneous because in fact that research did not lead to any result worthy of interest? The harsh criticism of the awarding of the Nobel prize to Bob Edwards comes to mind, a Nobel, it is said, gained at the price of the sacrifice of thousands and thousands of embryos; how many of the parents of the six million children born using MAP belong to the category of good Christians but have overlooked the extermination of future human beings in order to find a remedy for their suffering as sterile couples? And yet, and I hope that someone will remember that, an analogy between frozen embryos and the cold death of the Jews in concentration camps has already been made by some Catholic theologian ("frozen embryos are human beings in an ice concentration camp" Elio Sgreccia, "The Corriere della Sera", 17/3/2004).

The current situation of research on *gene editing* is in many respects the same, and we must add to this the fact that our country is considered by many European bioethics as the cradle of hypocrisy: we ban (and I quote an old English friend of mine) research on "national" embryos, but not on "European" embryos that we import with no shame, we are looking from the window with condescension pontificating and criticizing, but in reality we are letting others do the dirty work, ready to impudently make use of the results.

A personal remark signed by Profs. Assuntina Morresi and Bruno Dallapiccola (also signed by Prof. Francesco D'Agostino)

It is worth reiterating here, as indeed we have done repeatedly during the preparation of this document that this is not a general opinion on research that destroys embryos, a subject that the NBC has already previously discussed and on which, since there is no news, at present, there is no need for further reflection[50].

This opinion stems from a specific question about a new genetic manipulation technique, a very stimulating question for the NBC: when it is licit to transfer an embryo modified using *gene editing* into the uterus? These are non-generic modifications, related to known diseases, related to regions of DNA that have pathogenic mutations, and which therefore have therapeutic purposes and must be considered legitimate also according to law 40 (article 13).

This is a new problem, compared to those so far addressed by the NBC, which could have overcome the stale and in this case superfluous opposition between laity and Catholics, leaving aside schemes that are inadequate to provide answers to the issues that current scientific research raises.

Unfortunately it was not possible, if not in part. In the text, in fact, there are not two different orientations on the same topic, but two opinions, on two different themes. One, concerning research that destroys embryos, an issue that has little to do with the question posed, but which seems to be a formula (a mantra?) to be repeated each time the opportunity arises, in view of declaring oneself in favour of any research. The other, finally concerns the specific question of *gene editing*, as agreed at the beginning of the work.

[50] National Bioethics Committee, *The Destiny of Embryos Resulting from Medically Assisted Procreation (MAP) and not Complying with the Conditions for Implantation,* October 26, 2007**,** http://presidenza.governo.it/bioetica/pareri_abstract/destino_embrioni_da_PMA_26102012.pdf.

The two opinions are so different that it was necessary to use a different lexis. Choosing to speak of “basic” research, that is, aimed at "increasing knowledge", as opposed to “clinical” research, that is, "applied", is functional to the objectives of the first of the two views illustrated. It serves to establish the existence of "pure" research, which aims to "improve basic knowledge" (distinct from "applied" research). Obviously, this is research that we can not say no to: those doing so would refuse to look inside the telescope, to go beyond the columns of Hercules, they would be rejecting knowledge and truth, and of course, also *virtute*.

In short, with such premises, the answer can only be positive. The approach is now obsolete and above all, unsuitable for the questions to which the opinion should try to provide answers, rather it reiterates the existence of the opposition (that even as far back as the past century seemed a bit dated) between laity and Catholics, faith and reason, and so on.

This is not the place to explain the long debate over the use of the dichotomy of basic research/applied research, strongly contested in specialized literature and which have been given different interpretations, especially in these times, when we think in terms, for example, of convergent technologies, translational medicine, and system medicine, and when even the boundaries between separate disciplines have disappeared.

What is being called into question are not linguistic expressions, but visions of science and its relationship with society, from which different models of *governance* and participatory models arise[51].

But even without entering into the merits of the aforementioned topic, it should be obvious to anyone that research that does not have as its goal the increase of knowledge would be a contradiction in itself.

However, this has been the main obstacle in the work of the drafting of our text on *gene editing*, overcome only when the known, reassuring and tested language to the point of being inadequate to give answers to the new ethical and scientific dilemmas - research for the purpose of knowledge – was inserted into the text to reach the known, reassuring and proven argument, that is to say, to reiterate the need for research that destroys embryos.

Shame that it was neither the agreed subject, nor the topic requiring urgent responses.

A descriptive terminology such as "in vitro" and "in vivo" research - used in the second hypothesis of the opinion - allows instead to address the specific problem, while adhering to the conditions of the experimentation under discussion in order to carry out an assessment of it. It is no coincidence that it is precisely the Oviedo Convention, the world reference for biomedicine, to use these two expressions for research involving human embryos[52].

It is hoped that this will be a stimulus for reflection and discussions that go beyond the distinctions we have mentioned. It should be borne in mind that without the reasonable determination of the Presidency of the NBC the opinion would never have seen the light, and this deserves recognition.

Sheila Jasanoff Pforzheimer, Professor of Science and Technology Studies at Harvard University's Kennedy School of Government, tackled the issue of

[51] See, for example: S. Jasanoff, *Technologies of Humility: Citizen Participation in Governing Science*, Minerva 2003, 41: 223-244, or the recent: V. Narayanamurti and T. Odumosu, *Cycles of invention and discovery - Rethinking the endless frontier*, Harvard University Press, 2016.

[52] Convention on Human Rights and Biomedicine (Oviedo Convention), Art. 18, and Additional Protocol on Biomedical Research (2005), art.2, para 2.

governance of new *gene editing* technologies, proposing a simile which we consider enlightening.

In the famous "My Fair Lady" musical, based on George Bernard Shaw's Pygmalion, Eliza Doolittle, the *cockney* florist, takes lessons from Prof. Henry Higgins, phonetic expert, to become a real lady. But the professor, while transforming her into an acceptable person for high society, does not want to control just how Eliza speaks, but also, and above all, how Eliza thinks: the "right" language must also carry with it the "right" arguments. And we're all with Eliza when she sings “Why can’t a woman be like me?”

This reference to the film and the relationship between Eliza and the professor appears very appropriately in *CRISPR democracy: gene editing and the need for inclusive deliberation*, a recent publication by Jasanoff, the lead author[53]. The happy association between the specific citation of the film and a certain approach to developments in biomedicine, referred, in this case, precisely to the new *gene editing* technique examined, plainly clarifies the sense of this additional personal remark: there are arguments which seemingly, in order to be admitted to public discussion, must necessarily follow tracks that have already been traced out using pre-determined lexicon and arguments. Language that by convention is considered "right" with arguments that by convention are considered "right". And this opinion on *gene editing* is an example of this: the very long gestation period of the document and the unusually harsh confrontation of opinions accompanying it were the outcome of the attempt of a different approach to the one expected when it comes to scientific research on human embryos. Jasanoff concludes by expressing the fear that the scientists of academies that organized the summit in Washington in 2015 (reported in the NBC opinion) are likely to be "the Henry Higgins of CRISPR democracy".

We can therefore only agree, once again, with Sheila Jasanoff: *"The rarefied reasons of science are essential to any good deliberation on gene editing, but it is to be hoped that the deliberative processes we design will be expansive enough to let the unbridled Cockney in the rest of humanity also sing and speak*".

[53] S. Jasanoff, J.B. Huribut, K. Saha, *CRISPR Democracy: Gene Editing and the Need for Inclusive Deliberation*, Issues Sci. Technol, 2015, 32 (1).