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The tsunami named CRISPR/Cas9

It is rare to witness a breakthrough technology that results in a multidimensional revolution in human health that also has impacts on animals and plants as well as biodiversity and the environment. The tsunami associated with the latest genome-editing techniques is most likely not comparable to anything we have known thus far. In addition, the technology is not only relatively easy to deploy, but also developing at a dizzying pace.

Targeting the genome to change the sequence of a gene and its pattern of expression is not new. But, without question, the use of CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 [1] represents a major technological revolution. Indeed, this is the latest advance of a technology that allows the specific addition, removal or modification of DNA sequences, summarized by the term 'genome editing' [2]. If the genome-edited sequence corresponds to a gene, then the amino-acid sequence of the protein encoded by the gene may then be altered and, in some cases, lead to changes in its activity and function and/or its location or lifespan. Conversely, genome editing may result in the correction of the defective function of a gene within a specific biological context.

CRISPR/Cas9 induces a double-strand break in DNA at selected sites in the genome of any cell of any species. In practice, guide RNA (gRNA) leads the DNA endonuclease Cas9 to a specific sequence with instructions to cut through the DNA strands. The gRNA must be complementary to the chosen target sequence and located next to a short DNA sequence motif called 'PAM' (protospacer adjacent motif) to allow Cas9 to bind to the target genomic locus. The cell may then use two different mechanisms to repair the break: non-homologous end joining (NHEJ); or homology-directed repair (HDR). If the objective is to knock down the targeted gene expression, it is sufficient to allow NHEJ to mend the break. However, as NHEJ is prone to error, the 'repaired gene' will most likely be mutated by the insertion and/or deletion (INDEL) of random nucleotides. If the objective is to correct a preexisting mutation, then the repair has to restore the 'normal' sequence. To do this, HDR requires the introduction of a template DNA sequence, thereby allowing the cell to repair the break by copying the template sequence. This also applies when introducing a mutation to mimic a gene variant.

Given this rather simple scheme, the imagination of researchers seems to be without limits, as many technical adaptations have already been made to, for example, simultaneously modify multiple targets, use a dead Cas9 molecule, drive the increased/decreased expression of a gene, or of a fluorophore [3], or develop a programmable CRISPR system (RCas9) capable of specifically eliminating the toxic RNAs causing dominantly inherited diseases such as myotonic dystrophy type 1 or 2 (DM1/2), Huntington's disease or *C9orf72*-linked amyotrophic lateral sclerosis (C9-ALS) [4].
Virtually all branches of the life sciences will soon be penetrated by the rapid development of genome-editing technologies. For humans, genetic changes in somatic cells, germ cells and embryos are clear targets for these new approaches. As for other animals, both livestock and laboratory animals are likely candidates for these new methodological approaches, and the environment and biodiversity will also be clearly among the potentially affected areas. Gene drive approaches where a CRISPR/Cas9 cassette is able to self-perpetuate, thereby rapidly spreading any genetic information

among all members of a given population, could be applied for pest control, although this should also raise concerns over its misuse. Of equal concern is that gene-editing technologies could be used for the development of genetic weapons of mass destruction.
Several academic institutions and ethics committees, including the US National Academy of Sciences (NAS)/National Academy of Medicine (NAM)[1] and, more recently, the European Academies Science Advisory Council (EASAC)[2], have addressed the ethical, legal and social aspects (ELSA) raised by these new genome-editing tools. Based on its own report published in December 2015, the INSERM ethics committee organized a meeting in Paris, on 16 March 2016, with a wide range of European stakeholders to reflect upon and foster responsible research using genome-editing technologies [5].
The first ethical concern is to assess the feasibility, efficacy and safety of genome-editing techniques so that the benefit-to-harm balance of any potential clinical application can be properly evaluated. Because we still lack complete control over CRISPR/Cas9 binding and NHEJ *vs* HDR intervention after cutting DNA, there is a need to evaluate on-target variants, any potential off-target (cuts at non-selected sites) effects and mosaicisms (partial cuts resulting in different daughter cells, some repaired and some not), as well as epigenetic effects, in both the short and long term. Before such studies are promoted, however, there is a need to define standardized methods. To this end, we called for the establishment of a European Steering Committee (ESC) of experts from a wide range of relevant disciplines as diverse as molecular and cell biology, ecology and a variety of the social sciences [5, 6]. In addition, the ESC should rely on an open and transparent discussion process, including representatives of patient organizations, ethics committees and the economic sector, as well as of the communications sector.
A major ethical question is the potential application of genome-editing techniques to humans embryos. France, along with many other member countries, ratified the Oviedo Convention of the Council of Europe, including its Article 13, which is relevant to germline genome editing [6]. The Article prohibits any genetic modification that might be heritable. However, we believe that now is the time for open discussion of a case-by-case analysis for a limited number of genetic disorders, such as Huntington's disease, which may be prevented by genome editing. Clearly, this is necessary for society to maintain its general confidence in science, and requires the appropriate oversight of any laboratory work or medical application of genome-editing techniques, especially those that are irreversible and permanent.
The field of neurology will be impacted by genome-editing techniques in multiple ways. It will allow us to rapidly and easily design cellular and animal models of human neurological and mental disorders with known genetic bases, including most neurodegenerative as well as neurodevelopmental disorders. The new techniques are also changing the game for gene therapy, with ongoing clinical trials of muscular dystrophies, for example.
Another ethical challenge is to control the present overhyping of genome-editing techniques. Making certain therapeutic promises may engender dystopian expectations. Thus, it is important to raise awareness of the clear distinction between the care/treatment of human diseases and human enhancement.
Finally, attention needs to be drawn to the philosophical concept that opposes the dynamism and plasticity of all living organisms by adhering to the inadequate idea of a human nature founded solely on biological determinism. To do this, we must also

[1] https://www.nap.edu/catalog/24623/human-genome-editing-science-ethics-and-governance
[2] http://www.easac.eu/fileadmin/PDF_s/reports_statements/Genome_Editing/EASAC_Report_31_on_Genome_Editing.pdf

foster more debates both within the scientific community and with the rest of civil society.

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