This response was submitted to the Call for Evidence held by the Nuffield Council on Bioethics on Genome editing between 15 May 2017 and 14 July 2017. The views expressed are solely those of the respondent.

**Nuffield Council on Bioethics Genome Editing - Call for written evidence**
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This response aims to briefly discuss the technical elements of CRISPR based genome editing that must be separately and comprehensively addressed in the context of current use in research, but with particular regard to future use in human therapies.

The open call for evidence states that " 'genome editing' does not refer to a particular technique or an existing area of research but, rather, to the *idea* of using molecular approaches to alter genes or gene expression in purposive ways, however imperfectly this may be realised through the techniques currently in use".

In this respect, the field of genome editing is the same now as it was before the advent of CRISPR/Cas9. It has the same questions, the same goals and the same desired outcome: the advancement of knowledge and a progression in healthcare. These can be considered in parallel or in trans across all areas of biomedical research.

CRISPR/Cas9 has surpassed the efficacy of all its predecessors in terms of ease of use, affordability and accuracy, giving us the potential to take unparalleled steps closer to these objectives. However, techniques currently used for gene editing - that have already been used for decades - have received nothing like the scrutiny that CRISPR/Cas9 has.

**What makes genome intervention techniques distinct in their relative accuracy?**

Understanding this requires a discussion of the technology used for each technique. To date, every facet of CRISPR based genome editing has been altered and improved in terms of its technical application as well as molecular understanding. This is a dynamic technology in which competition and continuing research have fuelled its ongoing improvement and accuracy. The following aspects of the published applications of this technology will be discussed along with a brief comment on the current capacity of change within these areas:

1. CRISPR RNA-guided endonucleases (Endonuclease activity)
2. Mode of delivery
3. Method of repair (non-homologous end joining (NHEJ) or homology directed repair (HDR))

**1. CRISPR RNA-guided endonucleases**

There are two classes of CRISPR-Cas systems (that can be further divided into six types and 19 subtypes[1]) which allow the guided recognition and the subsequent cleavage at a target site. Class 1 effectors use multi-protein complexes and require several Cas proteins. Class 2 effectors, such as Cas9 and Cpf1, use single-component effector proteins. There is huge diversity in both the structure and the mechanism of these nucleases. It is important to distinguish between the classes because they have different editing abilities, and should be applied for the most appropriate editing event or target sequence. Further, they have recognised (and somewhat charactarised) differences in terms of off-target cutting events.

The majority of research to date has been done using the class 2 (type II) effector Cas9* however, Cpf1 (type V) has several advantages over it, depending on the sequence being cut. Firstly, Cas9, which in its natural form requires two RNAs, generates cleavage products with blunt ends, which are less easy to work with as a DNA sequence could be inserted in either end. Cpf1 makes staggered cuts that generate a 5′ overhang, which improves the precision of DNA insertions.
Secondly, unlike Cas9, Cpf1 cuts at a site distal to the gene, preserving the seed region. This is essential for target recognition if future editing is required.
Thirdly, Cpf1 has a T-rich protospacer-adjustment motif (PAM) suited to editing AT-rich DNA, whereas Cas9 has a G-rich PAM so the appropriate endonuclease should be applied for a particular sequence in question.
Lastly, because Cpf1 is smaller and does not require a tracrRNA, it may be easier to deliver to cells[4].

Ongoing fundamental research into microbial genomes has led to the discovery of other class II members of RNA guided (and DNA guided**) systems, such as C2c2, which has the ability to edit RNA. The increase in the number of systems leads to an increase in (PAM) sites available for editing, making more types of gene editing possible. The discovery of novel class 2 effectors could thus enhance the application of CRISPR systems to genome engineering.

The most disputed aspect of genome editing is the risk of "off-target cuts" or unintended mutagenesis which presents a clinical challenge and fuels ongoing ethical objections. Several developments, however, have led to major reductions in the level of off-target or unintended cutting events ranging from the use of altered Cas9 variants and the discovery of novel nucleases to the use of truncated guides.

* It is important to clarify which Cas9 homolog and protocol was used in a particular set of experiments as later work has shown that targeting DNA using a shorter strand of 'guide RNA' to direct the Cas9 enzyme reduces errors[2]. Furthermore, engineering of the Cas9 enzyme in the part of the protein that contacts the DNA target improves editing accuracy compared to the unaltered form of Cas9.[3]

**Controversy still exists over the genome editing ability of Natronobacterium gregoryi Argonaute (NgAgo) in human cells[5].

## 2. Mode of delivery

Both physical methods of delivery (such as electroporation, microinjection, laser) as well as vector-based delivery (viral and non-viral) are employed in the delivery of CRISPR machinery to cells or tissues. Optimising nuclease delivery aims to ensure both a high level of nuclease activity and low level of cytotoxicity. Specifically, the amount of nuclease must reach a critical threshold for the repair rate to allow a reasonable level of gene correction. However, high levels of nuclease can be cytotoxic.
The ability to minimise toxicity and deliver optimal amounts of nuclease into cells can pose a major challenge to nuclease-based therapies. Traditional viral and transfection-based delivery approaches, such as nucleofection, are each associated with significant drawbacks such as size limitations and immunogenicity.
Depending on the intended use, different systems require specific protocols and nucleases for efficient delivery of these CRISPR components. This ranges from

CRISPR expression systems used in research (which allow for both transient and stable expression of endonuclease) to targeting cells or embryos *in vivo* for both research and therapy. Choosing an appropriate delivery method and expression system means the difference between short acting alteration or a longer, more stable and integrating effect.

**3. Method of repair:** non-homologous end joining (NHEJ) or homology directed repair (HDR). In the absence of a repair template, as in the eukaryotic setting, the non-homologous end-joining (NHEJ) pathway generates insertions and deletions during double-stranded break (DSB) repair which can result in the loss of DNA within the region of edit. However, in the presence of a DNA template with homology to the sequences flanking the DSB location, homology-directed repair (HDR) can seal the DSB in a predictable manner less prone to error.

Genes interact with each other within and across each of these stages, so interchange between any of these individual steps can have a dramatic effect on the outcome of a genetic edit. While CRISPR has made gene editing "fast and simple", successful editing hinges upon a multitude of technical minutiae. Not all edits are created equally, therefore, in the scenario that applicable treatments do not have the desired effect in future, care should be taken not to question the entire technology. Instead each aspect of the treatment a particular research group uses - e.g. guide design, dosage, insertion locus, nuclease-directed cut and repair mechanism - should be examined.

**What conventional moral principles do genome-editing challenge?**

Like any treatment, clinically applied genetic engineering aims to avoid negative and untargeted effects, but we must also ask what is an acceptable error rate?

CRISPR has become a victim of its own success, providing potential solutions faster than health policy can be created. Unprecedented media attention has questioned the ethics of using this ground-breaking technology. However, human genome-editing treatments have already been carried out using both transcription activator-like effector nucleases (TALENs) and zinc finger nucleases (ZFNs), despite the major challenges these techniques present, such as cost, time and inaccuracy.

One argument for delaying the clinical use of CRISPR is that because of the rapid incremental improvements in the technology, further improvements are yet to be made. CRISPR is still in its infancy, but it is already more advanced and more accurate than techniques currently available. This begs the question "When will good, be good enough?".

Regarding arguments about the extent of off-target effects, many broadly used clinical interventions have well-characterised, unwanted side effects. Cancer treatment for example, involves a blunderbuss of radiation therapy, which can produce a range of undesirable effects, including the alteration (or destruction) of germ cells from the

patient. Future CRISPR treatments will likely be far more specific than current treatments in most clinical areas.
To give an example from the field of fertility treatment, intra-cytoplasmic sperm injection (ICSI) accounts for 70-85% of all IVF cycles. When sperm from genetically infertile males is used for this procedure, male offspring generated are guaranteed lower virility and will themselves be dependent on fertility treatment.

While somatic cell treatment is more broadly accepted, germ line modification i.e. altering the genome of future generations, rightly raises far more ethical concerns. For this reason, better understanding is needed to predict or eliminate potential harmful off-target effects. In order to do this, legislation should allow research on human embryos to be done more easily. A beneficial by-product of this will be a better understanding of human development, which will result in better clinical treatments.

The Hinxton Statement outlines that there are only three sources of human embryos considered for use in genome editing research: "nonviable embryos left over following *in vitro* fertilization; viable embryos left over following *in vitro* fertilization; and embryos created specifically for research[6]." None of those options is without problems. In discarded and non-viable embryos, the risk of mosaicism is high and the unknown mutation rate is higher. This makes it harder to evaluate the efficacy of gene editing in a given cellular context because the origin of deleterious effects is unknown.
Producing embryos for research is considered immoral, but creating embryos for IVF treatments is not. The average number of eggs fertilised per IVF cycle versus the average number that are implanted should be used as a benchmark figure for those alarmed by the use of a human embryo for research. Perhaps this would highlight that it is better to *use* a human embryo than waste it.

**"What obligations do governments have towards society to ensure 'safe' science or otherwise to shape the scientific research and development?"**

Gene modifications can be achieved within as little as one to two weeks from target design. In terms of shaping research and development, resources for cataloguing the vast quantities of data CRIPSR generates are sorely needed to encourage and facilitate collaboration and knowledge sharing. One such rare resource is CrisprGE: a dedicated repository-containing total of 4680 genes edited by CRISPR/Cas approach[7]. Allocations of realistic funding in all areas across this field are essential to achieve this.

To safeguard society, ensure public trust and to counteract the hysteria that harmful inaccurate publicity can cause, two actions will be critical:
- Transparency about progress in the field of gene editing and problems that may be discovered.
- Frequent and clear reassurance of the public of the existence and strong enforcement of legislation that prevents the field of gene editing moving from treatment of disease towards creating so-called "designer babies".

References:

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