Chapter 13
Safety and Security Risks of CRISPR/Cas9

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Abstract This case study looks into recent developments with regard to the CRISPR/Cas9 and other novel genome editing technologies that are becoming widely available thanks to their low costs and modest technological requirements.

Keywords Biosafety · Biosecurity · CRISPR/Cas9
Responsible research and innovation

Genome editing allows the specific modification of a genome; genes are modified within their respective location in the genome, making the changes often indistinguishable from natural mutations. Developments of this technology such as the use of gene drives, where specific genes are spread within populations, or the use of viral vector systems, are enabling additional applications in environmental engineering and disease treatment. There are substantial individual and societal benefits from applying genome editing; nonetheless the technology also poses significant risks to individuals, society as a whole and the environment.

The central focus of this case study is on the unresolved ethical issues related to safety and security that pose both short-term and long-term challenges to international research partnerships. As such, the case study focuses not on a single incident but on the risks in the proliferation of a new and very powerful technology at a time when accepted and tailored ethical and legal frameworks at the international, national and local level are missing.

In the case study two areas of *safety* risks are mapped and existing governance approaches described: first, risks to humans, for example in relation to therapeutic applications of genome editing; second, risks to the environment in relation to the use of genome editing on animals, plants and microbes. In addition, two aspects of *security* risks are also assessed: first, the creation of harmful agents relevant in the

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bioweapons context; second, human enhancement in a military context and its medium- and long-term implications for international security.

It is concluded that the rapid emergence of high-risk safety and security applications of genome editing challenge not only today's safety and security risk assessment but also existing governance tools. In addition, the absence of international standards of governance may result in safety- and security-sensitive experiments being transferred to countries with less stringent oversight, which will have serious implications for trust in international research.

Area of Risk of Exploitation

The key area of risk relates to the exploitation of international inconsistencies in biosafety and biosecurity with regard to the governance of genome editing experiments. These inconsistencies create an environment where risky experiments might be carried out in countries with no legal framework (European Commission nd), or in countries where, although legal frameworks exist, their implementation cannot be achieved due to limited resources (Dickmann et al. 2015). This undercuts established European standards of safety and security, while at the same time, due to the nature of some of these experiments, potentially affecting safety and security in Europe itself (Defensive Drives 2015).

Analysis

In everyday life, the terms "safety" and "security" are often used interchangeably. Here "safety" denotes the protection of humans, animals, plants and the environment from unintentional harm, whereas "security" relates to intentional harm (e.g. in a military context). This case study on genome editing focuses on the safety and security implications in four concrete experimental settings that have either been used in laboratories already, or are well within the range of existing technological capacities. These experimental settings are:

- the use of genome editing in human inheritable disease, infectious disease and cancer treatment and human enhancement
- the use of genome editing in creating novel pathogenic organisms
- the use of genome editing in environmental engineering and disease vector eradication
- the use of genome editing in agriculture

The controversy surrounding the publication of a research paper applying genome editing technologies to human embryonic stem cells has brought to the attention of the international scientific community the varying international governance approaches regarding such research. Since then a broad discussion has emerged on how to use this technology in an ethically sound way (Cyranoski 2015:272; Lanphier and Urnov 2015:411; Callaway 2016:16).

Although many of these discussions focus on the moral status of a human embryo and the permissiveness of human germ-line enhancement, it has become generally accepted that a common ethical issue is whether or not genome editing can be carried out safely and securely.

The safety aspect was highlighted very early on in the discussion as a critical limitation that would need to be resolved before any application of genome editing on humans or release into the environment could take place (Akbari et al. 2015). The security aspect, on the other hand, only recently gained attention when leading governmental officials identified genome editing as a national security threat (Oye et al. 2014).

Resolving the major safety and security concerns of genome editing is therefore of general importance, not only as a prerequisite for a reasonable discussion of the potential benefits, but also to foster trust among stakeholders in international collaborative research.

Genome editing has huge potential in human inheritable disease treatment and human enhancement. Research here relates to the treatment of various genetic disorders, infectious diseases and cancer. Recent examples that are currently undergoing safety testing in clinical trials are the use of somatic gene therapies involving immune cell modifications to treat cancer (Reardon 2016), CRISPR-based approaches to treating HIV (Reardon 2014) and the proof of principle of genome editing in the treatment of heritable diseases such as Duchenne muscular dystrophy (Mendell and Rodino-Klapac 2016). Key safety concerns in this area have been the number of off-target changes, mosaicism and potential epigenetic effects (Next-generation genome editing 2015). These are not new safety concerns, but have also been encountered in other gene therapeutic approaches. The existing step-wise approach applied in clinical studies should therefore be sufficiently robust to identify, assess and govern such risks.

There is a fluid relationship between genome editing as employed in heritable disease treatment and its use for human enhancement (Ishii 2015; Cox et al. 2015). Genetic human enhancement has substantial security implications. In certain countries, approving the use of genome editing for this purpose (e.g. IQ and physical endurance) would have far-reaching military and economic security implications at the national and international level. These security risks need to be included in risk benefit assessments of human enhancement based on genome editing.

Certain genome editing techniques open the possibility for the development of a new class of infectious pathogenic organisms. A recent example has been the creation of cancer models in mice, where the cancerous mutation was introduced through genome editing using viral vectors – in essence transforming cancer into a transmissible infectious disease (Chiou et al. 2015). This creates novel safety risks that will need to be included in biosafety oversight schemes. In addition, such work has the potential to create new generations of biological and chemical weapons which might not be detectable by current diagnostics.

The use of genome editing in environmental engineering has been discussed in the context of pest control, with new ways to eradicate agricultural pests (Huang et al. 2016; Leftwich et al. 2016), as well as that of disease eradication. For example, gene drive systems are being developed to eradicate malaria (Gantza et al. 2015), and contemplated for the eradication of the Zika (Hegg 2016) arthropod vector. Key safety concerns relate to the environmental harmfulness, controllability and reversibility of such environmental interventions. Key security concerns relate to their potential use as socio-economic and environmental weapons.

The use of gene drives in an environmental context creates novel risks for both safety and security, which are not restricted by national boundaries. Current national and international risk management approaches to biosafety and biosecurity are incapable of mitigating these risks adequately.

The use of genome editing in agriculture for breeding purposes in plants and animals (Sovová et al. 2016) creates unique and novel challenges to biosafety and biosecurity. Key safety concerns relate to the outbreeding and spread of these new varieties into natural populations, the detectability of these new variants (Breeding Controls 2016) and challenges to established coexistence provisions (Ledford 2015).

Below are quotations from leading researchers that address some of the relevant issues on biosafety and biosecurity (all quoted in Ledford 2015):

Leading Researchers	Quotes
James Haber – on the issue of off-target effects:	These enzymes will cut in places other than the places you have designed them to cut, and that has lots of implications.
Jennifer Doudna – on the biosafety and biosecurity of an experiment creating a human cancer model through a CRISPR-engineered virus:	It seemed incredibly scary that you might have students who were working with such a thing. … It's important for people to appreciate what this technology can do.
George Church – on the safety risks of gene drives in relation to the environment:	It has to have a fairly high pay-off, because it has a risk of irreversibility – and unintended or hard-to-calculate consequences for other species.
Jennifer Kuzma – on the detectability of genome-edited GMOs in nature:	With gene editing, there's no longer the ability to really track engineered products. It will be hard to detect whether something has been mutated conventionally or genetically engineered.
Kenneth Oye – on governance:	It is essential that national regulatory authorities and international organizations get on top of this — really get on top of it.

Recommendations

There are four levels on which recommendations can be made to avoid the exploitation of safety and security weaknesses in genome editing in the future.

Technical Level

- Reduce off-target effects, mosaicism and epigenetic effects through further research in higher fidelity and better understanding of genome editing technologies.
- Use safe virus systems or alternative less risky vector systems to transfer genome editing tools.
- Develop reversal gene drives in parallel that can undo the effects of gene drives.
- Provide technological assistance (e.g. detection capacities for modified organisms) in implementing international obligations such as the Cartagena Protocol.

Containment Level

- Ensure adequate biosafety risk classification and implementation of adequate containment measures in biosafety-sensitive genome editing experiments.
- Develop "molecular containment" approaches when working with genome-edited high-risk pathogens.

Governance and Oversight level

- Provide international guidance or amend existing guidance documents on biosafety and biosecurity to cover risks from genome editing.
- Map the status of existing biosafety and biosecurity legislation as well as its practical implementation in countries carrying out genome editing experiments.
- Include stakeholders (e.g. funding institutions, research institutions, researchers) in the responsible governance of research involving genome editing.

International Standardization

- In case of gaps in legal oversight, develop international codes and guidelines for safe and secure work in genome editing.

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