University of Pittsburgh Institutional Biosafety Committee
Recommendations for Biosafety Level Assignment for
Gene Editing Technology

Approved February 8, 2016
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Background

Several endonucleases are available to edit mammalian genes within cells, including murine embryos to create genetically modified mice. These include zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeats/CRISPR-associated (CRISPR/Cas) system. ZFNs and TALENs rely on sequence-specific DNA-binding motifs fused to non-specific endonucleases. The DNA-binding domain targets the nuclease to a gene of interest and the nuclease produces double-strand breaks on either side of the target sequence, leading to disruption of the gene. More recently, Cas proteins were identified in bacteria that utilize a single guide RNA (sgRNA) that is complimentary to a gene of interest and targets the Cas nuclease to the gene. Like ZFNs and TALENs, Cas proteins produce a double-stranded DNA break where disruption of the gene can occur. Modifications to the Cas enzyme and guide RNA system have been made in which Cas proteins can modify single bases specifically, produce single-strand DNA breaks, or increase/decrease transcription of genes.

There are biosafety concerns in the use of these technologies. Expression of ZFNs, TALENs, and CRISPR/Cas with sgRNAs can lead to permanent disruption of genes in cells. They can be introduced into cells by transfection of plasmids (or ribonucleoprotein complexes for CRISPR/Cas and sgRNAs) or by transduction with replication-defective viral vectors, including retroviruses, lentiviruses, adenoviruses and adeno-associated viruses (AAV), that are readily obtained from commercial sources.

All three gene editing endonucleases have been shown to have "off-target" effects, in which the wrong gene could be disrupted. This can lead to unintended phenotypes in a cell. In the case of the CRISPR/Cas system, sgRNAs targeting a gene in one species could cross-react in another species when high homology between genes exists.

Improper use of viral vectors could lead to accidental infection of laboratory personnel. Of note, retroviruses or lentiviruses integrate into the host genome, which is stable and permanent for the life of the cell. In some systems, re-introduction of packaging and viral entry plasmids into these cells could lead to production of new virus particles that are released from the cell. Furthermore, retroviral and lentiviral genome integration is associated with unchecked cell growth due to integration in or near host cell genes involved in cell proliferation.

NIH opinion

The NIH makes clear that the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)*, *Section IV-A (Policy)*, is a living document and as such

cannot be complete due to developments of new technologies and reagents. Thus, the responsibility is on the institution and those associated with it to adhere to the intent as well as the specifics of the *NIH Guidelines*.

IBC Rationale

The University of Pittsburgh IBC discussed safety concerns based on current knowledge with respect to this fast-evolving technology and in keeping with all appropriate institutionally recognized safety guidelines and regulations. If new information is discovered that substantiates changes, the committee will convene discussion to provide the most appropriate safety guidance to the research community.

In accordance with current applicable Occupational Safety and Health Administration (OSHA) regulation 29 CFR 1910.1030, BSL-2 containment is recommended for activities involving all blood-contaminated clinical specimens, body fluids, or unfixed tissues/cells from all humans. The IBC has maintained a long-standing requirement of the use of BSL-2 containment or “Universal Precautions” practices for research activities involving unfixed human tissues or cells, including untested or uncertified human cell lines that may be available commercially. This tradition is consistent with the efforts by the Department of Environmental Health and Safety in ensuring occupational health and safety tenants.

Institutional Biosafety Committees are charged with ensuring that institutions adhere to the spirit and intent of the *NIH Guidelines*, including *Section II-A-3*, which specifies institutional responsibility for a “Comprehensive Risk Assessment” of research activities. Because the OSHA Bloodborne Pathogen Standard is included in the assessment of risk, the IBC has agreed with the recommendations for BSL-2 or “Universal Precautions” when working with gene editing endonucleases using transfection of plasmids or ribonucleoprotein complexes that are incapable of directly entering cells. Viral vectors that encode either gene targeting endonucleases (e.g., TALENs or ZFNs) or CRISPR/Cas together with one or more sgRNAs must be generated and used at BSL-2+ containment. If CRISPR/Cas and the sgRNA(s) are expressed using separate viral vectors, BSL-2 downgrade can be granted.

IBC Guidance

The University of Pittsburgh IBC will apply the following criteria for determining the appropriate biosafety containment and handling of research involving ZFNs, TALENs, and CRISPR/Cas:

- Use in cell culture (*in vitro*)
- Viral vs. non-viral expression method (viral transduction vs. non-viral transfection)
- Expression of CRISPR/Cas and sgRNAs separately
- Administration into live animals

ABSL-1: Recommended for non-viral administration into animals

The IBC will consider the expression of ZFNs, TALENs, and CRISPR/Cas from plasmids or ribonucleoprotein complexes at ABSL-1:

- Administration of plasmids encoding ZFN, TALEN, or CRISPR/Cas into animals without human cells or tissues
- Administration of ribonucleoprotein complexes consisting of CRISPR/Cas protein and sgRNAs into animals without human cells or tissues

BSL-2: Recommended for non-viral expression in cells

The IBC will consider the expression of ZFNs, TALENs, and CRISPR/Cas from transfection of plasmids or ribonucleoprotein complexes at BSL/ABSL-2:

- Transfection of plasmids encoding ZFN, TALEN, or CRISPR/Cas into cells
- Transfection of ribonucleoprotein complexes consisting of CRISPR/Cas protein and sgRNAs into cells (see https://www.ncbi.nlm.nih.gov/pubmed/29995861)

BSL/ABSL-2: Recommended for separate expression of CRISPR/Cas and sgRNAs from sequential replication-defective virus transductions

The IBC will consider the expression of CRISPR/Cas and the sgRNA(s) encoded in separate replication-defective viral vectors delivered more than 72 hours apart at BSL/ABSL-2:

- Transduction of CRISPR/Cas-expressing cells with a replication-defective virus (e.g., retrovirus, lentivirus, adenovirus, AAV) encoding sgRNA
- Transduction of cells first with a replication-defective virus encoding CRISPR/Cas* and transduction > 72 hours later with a replication-defective vector encoding sgRNA**
- Administration of a replication-defective virus encoding sgRNA into genetically modified animals expressing CRISPR/Cas9

BSL-2+/ABSL-2: Recommended for expression of TALENs, ZFNs, or CRISPR/Cas together with one or more sgRNAs from viral vectors

The IBC will consider the use of TALENs, ZFNs, or CRISPR/Cas together with one or more sgRNAs using replication-defective viruses for use at BSL-2+/ABSL-2:

- Transduction of cells with a replication-defective virus (e.g., retrovirus, lentivirus, adenovirus, AAV) encoding ZFN
- Transduction of cells with a replication-defective virus (e.g., retrovirus, lentivirus, adenovirus, AAV) encoding TALEN
- Transduction of cells with a replication-defective virus (e.g., retrovirus, lentivirus, adenovirus, AAV) encoding both CRISPR/Cas and one or more sgRNAs
- Administration of a replication-defective virus encoding ZFN, TALEN, or CRISPR/Cas9 plus sgRNAs into animals

* Examples of lentiviral vectors expressing only CRISPR/Cas9 are:

- lentiCas9-Blast (Addgene #52962)
- lentiCas9-EGFP (Addgene #63592)
- pRCCB-CMV-Cas9-2A-Blast (Cellecta #SVC9B-VS)
- pRCE2B-EFS-Cas9-2A-Blast (Cellecta #SVCE2B-VS)
- LentiArray Cas9 Lentivirus (ThermoFisher #A32064, A32069)
- MSCV-hspCas9-T2A-Puro Lentivector (System Biosciences #CASLV120PA-1)
- EF1a-hsaCas9 AAV (System Biosciences #CASAAV200PA-1)

** Examples of vectors expressing only sgRNAs are:

- pLenti SpBsmBI sgRNA Puro (Addgene #62207)
- pLenti SpBsmBI sgRNA Hygro (Addgene #62205)
- LentiArray CRISPR gRNA Lentivirus (ThermoFisher #A32042)
- EF1-RFP-U6-gRNA (System Biosciences #CASAAV300PA-1)