University of Pittsburgh Institutional Biosafety Committee Recommendations for Biosafety Level Assignment for "Gene Drive" CRISPR/Cas-9 Technology

Approved February 8, 2016

Background

A new-comer to the gene editing technology field is Clustered Regularly Interspaced Short Palindromic Repeat or CRISPR. This technique uses a system of adaptive immunity that bacteria use, and has been expanded to disrupt or alter genes in all types of cells. Expression of CRISPR/Cas9 and guide RNAs from retroviruses or lentiviruses, allows for integration into the host genome, which is stable and permanent for the life of the cell. The IBC has discussed safety issues of the potential for disruption of genes not targeted by guide RNA sequences which are known as "off-target" effects.

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, nor able to foresee the utilization of new genetic manipulation that could enable work to be accomplished faster, more efficiently or at larger scale. 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 the current state of knowledge with respect to this fast-evolving technology and in keeping with all appropriate institutionally recognized safety guidelines and regulations. If new knowledge 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 on the commercial market. This tradition has been consistent and complimentary to the efforts by the Department of Environmental Health and Safety with 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 the 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 other potentially infectious materials.

IBC Guidance

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

- Use in cell culture (*in vitro*)
- Propagation method (viral transduction vs non-viral transfection)
- Administration into live animals
- Administration into human subject participants

BSL/ABSL-1: Recommended for non-viral, non-human cell use

The IBC will consider the use of CRISPR in cell culture work viruses for use at BSL/ABSL-1:

- Transfection of cells in culture, except for human-derived cells

BSL/ABSL-2: Recommended for viruses or use in human cells

The IBC will consider the use of CRISPR in cell culture work viruses for use at BSL/ABSL-2:

- Transfection in human-derived cells
- Transduction of cells in culture