Marshall University Institutional Biosafety Committee (IBC)
Guidance for rDNA applications based on lentiviral vectors

Approved by the MU IBC on 02/10/2016

I. Introduction

This document provides essential information for research involving the use of lentiviral expression vectors. Research involving lentiviral vectors fall under the NIH recombinant DNA (rDNA) Guidelines. Therefore, all research Investigators who anticipate use of lentiviral vectors must submit an rDNA/infectious agent application and receive IBC approval before working with these vectors. The work must be conducted under Biosafety Level 2 (BSL-2) or enhanced BSL-2 work practices. The IBC will determine the final Biosafety Level and or Animal Biosafety Level (ABSL) for work with lentivirus based on risk assessment and in accordance with regulations specified in the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines; http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines), the RAC Guidance Document on Biosafety Considerations for Research with Lentiviral Vectors (http://osp.od.nih.gov/sites/default/files/resources/Lenti_Containment_Guidance_0_0.pdf), the CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL) document, (http://www.cdc.gov/biosafety/publications/bmbl5/) and guidelines contained in this document. A lab-specific protocol for safe handling of lentivirus must be provided along with rDNA application. The lab head or designee must provide all lab members with training in the lentiviral safe handling and share any guidance from IBC.

II. Lentivirus

Lentivirus is a retrovirus that includes several human pathogens including the Human Immunodeficiency Virus (HIV), Simian Immunodeficiency Virus (SIV) and Human T-lymphotropic Virus (HTLV). Laboratory acquired infection can occur through percutaneous or mucocutaneous exposure of human and non-human primate bodily fluids: blood, semen, saliva, tears, urine, cerebrospinal fluid, amniotic fluid, breast milk, cervical secretion, and tissue.

Protocols that involve production of research-laboratory scale quantities of HIV or SIV, manipulating concentrated virus preparations, and conducting procedures that may produce droplets or aerosols, must be performed with Biosafety Level 2 (BSL-2) with additional safety practices and precautions specific to work with lentivirus. **This is referred to an Enhanced BSL-2** and will be described in Section IV of this document.

Because research with wildtype HIV-1, HIV-2, HTLV-1, HTLV-2, and SIV involving large scale volumes or preparation of concentrated virus must be conducted under BSL3, these types of research are not allowed at Marshall University.

III. Lentiviral Vectors

IIIA. General description of generations of vectors

Lentiviruses can infect not only dividing and non-dividing host cells, because the intact virus can pass through the intact membrane of the nucleus of the target cell nucleus. Lentiviral

vectors can be used to provide highly effective gene therapy as they can provide long-term expression of the vectored transgene in target cells. First-generation lentiviral vectors were manufactured using a single vector packaging system that contained all HIV genes, except the envelope (env) gene, in one plasmid. In the second-generation system, five of nine HIV-1 genes were eliminated, leaving the two genes which encode structural and enzymatic components, and the two genes for transcriptional and posttranscriptional functions.

A four plasmid vector system is used in a third generation lentiviral vector. By splitting the vector system into 4 plasmids (3 helper plasmids and 1 containing the expression vector and the transgene), the third generation lentiviral vector system offers advantages over the previous generations because the number of recombination events required to form a complete replication-competent virus increases, thereby reducing the possibility of making a replication-competent viral particle.

The MU Institutional Biosafety Committee (IBC) will determine the BSL of work involving the use of Lentiviral vectors based on BMBL, NIH Guidelines, RAC Guidance documents and the lab specific risk assessment. This risk assessment will evaluate the potential for generation of replication-competent lentivirus (RCL) and the potential for oncogenesis from the transgene among other risk factors.

Work with lentivirus in the vector form must be conducted under either BSL-2 or enhanced BSL-2 work practices depending on the risks associated with research protocols.

IIIB. Risks of lentivirus vectors:

The major risks to be considered for research with HIV-1 based lentivirus vectors are

- the potential risk of insertional mutagenesis as a result of an exposure
- the nature of the vector system and potential for generation of replication-competent lentivirus (RCL)
- the nature of the transgene insert (e.g. known or potential oncogenes and immunoregulatory genes; known oncogenes or genes with high oncogenic potential may merit special care)
- the vector titer and the total amount of vector
- the inherent biological containment of the animal host, if relevant)
- negative RCL testing (see section VII below)
- the exposure potential for HIV positive individuals whose native virus may recombine with or complement the vector

Some risks can be mitigated by the nature of the vector system (and its safety features) or exacerbated by the nature of the transgene inserted into the expression vector.

IV. Laboratory Practices and Containment Equipment (enhanced BSL-2 practices)

In addition to complying with the corresponding facility and work practice containment as specified in the BMBL Section IV (“Laboratory Biosafety Level Criteria”) and the NIH Guidelines, the following work and containment practices should be considered for inclusion in the lab-specific

protocol. We use the term “Enhanced BSL-2” as a general term where additional lab practices are included with the set of BSL-2 practices. The actual enhanced BSL-2 work practices will be specialized for each lentivirus and lentiviral protocol.

The following Enhanced BSL-2 practices should be considered for each protocol.

- The principle investigator must provide training in the safe handling of lentiviral vectors. This training must be include review of the lab-specific protocol with technical staff and observation of staff to determine if the worker is compliant and proficient in the protocol.

- No work with Lentivirus is permitted on the open bench. A Biosafety Cabinet must be used for all manipulations including (but not limited to):
    a. pipetting
    b. harvesting infected cells for RNA/DNA/proteins
    c. loading and opening containers
    d. initial delivery of vector in animal hosts
- Reduce risk of exposure by reducing potential of replication competent lentivirus using one of the following methods:
    a. Segregate the lentivirus into vectors and packaging replication functions onto four or more plasmids. The use of HIV – 3rd Generation Packaging or HIV – 4th Generation Packaging Systems are recommended.
    b. Use non-native Env or a heterologous coat protein (e.g. VSV-G) in place of the native HIV-1 envelope protein. (However, the use of the certain coat proteins, such as VSV-G, may broaden the host cell and tissue tropism of lentivirus vectors, which will be considered in the overall risk and biosafety assessment by IBC.)
    c. Removal of genes essential for replication such as TAT.
- Labels must be placed to indicate each area where Lentivirus is used or stored including, but not limited to, biosafety cabinets, incubators, refrigerators, laboratory entrance doors, etc.
- All vacuum lines must be fitted with a HEPA filter (e.g. “Vacushield J” in-line hydrophobic filter, Product #4402 from Gelman Sciences).
- All procedures involving manipulation of the virus or viral vector or infectious materials must be conducted within a certified biosafety cabinet or other physical containment device.
- Centrifugation must be done in screw-capped centrifuge tubes that are placed inside sealable rotor buckets and using rotors with screw-top lids. Rotors must be loaded and unloaded in a Biosafety cabinet.
- Strict attention to surface decontamination
- Limit access to lab when lentiviral work is being performed
- Avoid needles and sharps where possible.
- PPE: Enhanced BSL-2 includes the use of the following personal protective equipment to reduce the potential for mucosal exposure, splash to the face, and exposure of hands:
    - Gloves should be used at all times when handling viral samples. Gloves should be pulled over the cuffs of disposable lab coats.
    - Disposable lab coats are preferred.

- Wrap around outer clothing when introducing vector into animals or performing necropsies. Lab coats are adequate for tissue culture manipulations.
- Goggles (not to be confused with safety glasses)
- N-95 respirator, to be used with concentrated titers and highly aerosolizing procedures outside of the Biological Safety Cabinet (contact EH&S for further information) or in ABSL 2 facility.
- Bloodborne pathogen training is recommended for all employees working with lentivirus and lentiviral vectors.

- Medical Surveillance:
- Personnel who are working with wildtype lentivirus should discuss serum banking with the Biosafety Officer prior to the start of work.

V. Animal Research with Lentivirus

When animals are infected with lentivirus/lentiviral vectors, an Enhanced Animal BSL-2 (Enhanced **ABSL**) protocol must be approved and used for the procedure. Concurrent approvals are needed from the MU Institutional Biosafety Committee (IBC) and the MU Institutional Animal Care and Use Committee (IACUC). Requirements for ABSL-2 containment facility and work practices are indicated in BMBL Section V. Vertebrate Animal Biosafety Level Criteria for Vivarium Research Facilities.

Some animals, such as wild-type mice, cannot support replication of infectious HIV-1. As a result, the potential for shedding of the virus from such animals is very low. Precautions must be taken not to create aerosols when emptying animal waste material and when washing down cages, or cleaning the room with pressure hoses so as to minimize the risk of autoinoculation by the lab personnel/investigator. If there is no expectation of infection, the site of inoculation has been thoroughly cleansed, and the bedding changed, it may be acceptable to consider reducing containment from ABSL 2 to ABSL 1 within a few days (the specific time period can be specified by the SDSU IBC, and may vary anywhere from 1-7 days depending on local and experimental considerations). It is strongly recommended by the Institutional Biosafety Committee that lab personnel be responsible for all Enhanced ABSL-2 animal husbandry practices following infection of the animal. After the allotted time (72 hours), the animals can be housed at ABSL-1 facility. Contact the Biological Safety Officer or the IBC Chair for additional chair for guidance Enhanced ABSL-2 practices.

Animals engrafted with human cells or animal hosts that are permissive for HIV-1 replication constitute a special case, in light of their potential to support replication of infectious HIV-1. Use of lentivirus vectors in these animals requires a continuous high level of containment at Enhanced ABSL-2. **In this case all animal husbandry practices will be conducted by the lab personnel/investigator following infection of the animal.** Animals should be housed and kept at Enhanced ABSL-2 until euthanized.

Special training must be given to all animal husbandry personnel on lentivirus, the hazards associated with the research, required practices and procedures and proper handling of bedding, cage washing, and all other husbandry materials associated with the experiment. Contact EH&S at (619) 594-2865 to schedule a group training.

VI. Risks associated with lentiviruses

The major risks to be considered for research with lentivirus vectors are

- potential for generation of replication-competent lentivirus (RCL), and
- potential for oncogenesis.

These risks can be mitigated by the nature of the vector system (and its safety features) or exacerbated by the nature of the transgene insert encoded by the vector.

Non-human lentivirus vectors (e.g. feline immunodeficiency virus) require the same level of safety as human lentivirus vectors, since these vectors have the potential to transduce human cells, and thus have the potential to cause insertional mutagenesis.

The virus is a slow infecting disease with the onset of symptoms occurring years after initial infection. The virus targets immune cells, leading to immunodeficiency.

Transmission of the virus is through bodily fluids so precaution must be taken when working with animals containing the virus or virus vector.

VII. Replication Competent Lentivirus (RCL) testing (excerpted from the RAC Biosafety Considerations for Research with Lentiviral Vectors)

"The FDA requires that lentiviral vector stocks used in human clinical trials be tested for RCL. Individual research laboratories conducting preclinical research often use only small volumes (e.g., a few milliliters) of lentivirus vectors expressing lower risk transgenes such as GFP. While these laboratories are not mandated to characterize vector stocks, such testing should be encouraged. However, RCL testing requires expertise with the appropriate assays and such expertise may not be available in laboratories that do not work regularly with infectious lentiviruses. In such laboratories, the use of a positive control may increase risk to the investigator as compared to use of the test material. **IBCs may make containment assignments without requiring such testing by undertaking a risk assessment that considers the nature of the specific vector system being used and overall past experience with the system."**

For all lentiviral work in humans, the MU IBC requires that assays for RCL must be performed.

VIII. Employee Exposure

Response to Accidental Exposure and reporting requirements.

VIIIA. Mucous membrane exposure from splash or aerosol

Rinse a minimum of 15 minutes in eye wash or flush with water. Notify the Principal Investigator or Laboratory Supervisor, who will immediately contact Diane Alcorn at 1-304-696-6285 and direct the exposed employee to appropriate medical treatment at Marshall Health Services. A workplace injury/illness form which can be found at http://www.marshall.edu/safety/files/2013/04/HR-SERV-FORM-31.pdf must be completed.

VIIIB. Needlestick, sharps exposure or non-intact skin exposure

Contaminated skin should be scrubbed for approximately 20 minutes using a 10% povidone iodine solution (such as Betadine®) and copious amounts of water. Notify the Principal Investigator or Laboratory Supervisor, who will immediately contact Diane Alcorn at 1-304-696-6285 and direct the exposed employee to appropriate medical treatment at Marshall Health Services. A workplace injury/illness form which can be found at http://www.marshall.edu/safety/files/2013/04/HR-SERV-FORM-31.pdf must be completed.

VIIIC. Decontamination of surfaces

The most effective germicides (with minimum 15 minute contact time) are:

- Freshly prepared 0.5% Sodium hypochlorite
- 2% Glutaraldehyde
- 5% Phenol

Lentivirus is sensitive to heat, detergents, and formaldehyde.

IX. Employee Right-to-Know

It is important that all lab personnel (even those not directly working with the virus) be informed and aware that lentivirus or lentiviral vectors are being used in the lab. The Principal Investigator or Lab Head must verbally notify lab members under his/her direction and post notices of the lentiviral work to all rooms where the work is being conducted.

Appendix 1: Examples of IBC Lentiviral Risk Assessment

(From the RAC document "Biosafety Considerations for Research with Lentiviral Vectors")

Example 1: *In vitro* study A

Use of a 4-plasmid derived lentivirus vector encoding siRNA against Lck in primary human T cells.

Considerations

1. What is the amount of vector to be produced? Low <100ml
2. What is the nature of the vector? 4-Plasmid System
3. What is the nature of the insert? Non-oncogenic insert

Tentative Risk Assessment = BSL 2

Example 2: *In vitro* study B

Use of a 2-plasmid derived lentivirus vector encoding luciferase in a human cell line (a549 cells).

Considerations

1. What is the amount of the vector to be produced? Low < 100ml
2. What is the nature of the vector? 2-plamsid System (non-commercial)
3. What is the nature of the insert? Non-oncogenic

Tentative Risk Assessment = BSL 2 + Lenti (**MU enhanced BSL2**)

Example 3: *In vivo* study B

Use of a 4-plasmid derived lentivirus vector encoding brain-derived neutrotrophic factor (BDNF) in mouse brain.

Considerations

1. What is the amount of the vector to be produced? Low < 100ml
2. What is the nature of the vector? 4-plamsid System
3. What is the nature of the insert? Non-oncogenic*
4. What is the nature of the animal host? Non-permissive for HIV-1

Tentative Risk Assessment = ABSL 2 + 7days, then downgrade to ABSL 1.

* Even though BDNF is a growth factor for neurons, it has no known oncogenic activity for skin or blood cells that might be the target of a potential needle stick. Hence, this insert would not automatically trigger a requirement for increase biocontainment.

References

Biosafety in Microbiological and Biomedical Laboratories
http://www.cdc.gov/OD/ohs/biosfty/bmbl5/BMBL_5th_Edition.pdf

NIH Guidelines for Research Involving Recombinant DNA Molecules
http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html

ABSA Alliance Lentivirus Vector Fact Sheet:
https://www.absa.org/pdf/LentivirusVectorFactSheet.pdf

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