Cornell University Institutional Biosafety Committee
Guidance on the Use of Lentiviral-Based Vectors

Overview

Third generation lentiviral vector systems (e.g., four plasmid systems) are widely used to transfer genes in cell culture systems and live animals (producing transgenic lines). Many of these systems are derived from HIV-1 (human immunodeficiency virus), the risks of which include the generation of replication competent lentivirus (RCL) and the potential for oncogenesis through insertional mutagenesis. A number of features are incorporated in the latest vector designs to enhance biosafety. These features include:

- Minimalist design of the vector backbone (number of HIV genes is reduced to three (*gag*, *pol*, and *rev*) and a substantially deleted env gene.
- Vector and packaging components are distributed on four or more plasmids (proteins supplied *in trans*) that contain very little, if any, overlap or homology
    - One of the three packaging plasmids encodes the heterologous coat protein, VSV-G, which holds no homology to HIV-1 envelope protein
- Deletion in the 3' LTR that results in "self-inactivation"
- Elimination of the TATA sequence, essential for replication of wild type HIV-1

The result of these enhancements is a vector that has little capability of producing replication competent virus. Although these systems are much safer, relative to older generation systems that separated viral components on two plasmids, the vectors can efficiently and effectively transduce human cells. This presents a risk for investigators.

Laboratory Applications

Lentiviral vectors must be handled, at a minimum, under biosafety level 2 (BSL2) containment and operating conditions to minimize the potential for autoinoculation.

- The IBC strongly recommends that investigators utilize commercial vector systems (e.g., vendors A, B)
- The IBC highly recommends that all vector handling, regardless of the potential for aerosol or droplet production, be conducted within the biosafety cabinet
- Limit the use of hypodermic syringes and needles and other sharps. Strongly consider the use of engineered sharps systems
- Use disposable gloves and a lab coat
- Routinely decontaminate work surfaces with a suitable disinfectant (e.g., 10% bleach)
- Change gloves frequently to minimize the spread of contamination and wash hands
- Treat all solid waste as regulated medical waste. Recommend that waste is pre-treated in an autoclave
- Decontaminate liquid waste with a disinfectant or in an autoclave before disposal down the drain
- Use sealed centrifuge tubes and rotors and load and open tubes and rotors in the biosafety cabinet

For more detail, refer to the biosafety level 2 checklist and the precautions outlined in the BMBL (Biosafety in Microbiological and Biomedical laboratories, 5th edition) and the NIH Guidelines.

Additional factors that can impact the risk assessment process are:

- Nature of the transgene. For example, special conditions may be required for vector gene inserts such as oncogenes, genes with high oncogenic potential, toxin producing genes, antagonist of a tumor suppressor gene, or some other cell cycle gene.
- Titer of the vector and the amount. Generally, as titer and amount increase, so does the risk of accidental exposure.

These additional risk factors will require that extra precautions be put in place. They include:

- double gloving
- solid front gown
- mucous membrane protection (e.g., safety glasses, face mask, or face shield), especially if conducting any procedures outside of the biosafety cabinet
- thorough review by the IBC

Testing of Vectors

At this particular time, testing of small preparations (<100 ml) of vector stocks is not required. Many laboratories may lack the expertise and resources to conduct thorough and meaningful testing. Also, the addition of a positive control (e.g., infectious HIV) to the testing procedure would significantly increase the risk for investigators. In the absence of testing, laboratories must use the latest generation systems (see above), the systems must be obtained from a reputable, commercial supplier, and the investigator must use BSL2 procedures and containment. Large volumes (several hundred mls and greater) of vector stocks may require testing at the discretion of the IBC.

The IBC highly recommends that investigators verify the identity of the plasmids obtained from other colleagues or institutions before these plasmids are used. Researchers can use sequence, restriction digest, or other suitable data to obtain this quality assurance. Investigators should submit the data to the IBC for review.

Older generation lentiviral vector systems (e.g., those systems with viral components separated on two plasmids) have fewer features to enhance safety and are more likely to produce RCLs. This can significantly increase the risk for investigators, especially in laboratories that do not specialize in gene vector applications. These older systems require enhanced containment, testing, and oversight. As a result, the IBC strongly discourages the use of these vector systems.

Applications in Mice

Wild type or immuno-competent mice cannot support the replication of infectious HIV-1. There are multiple blocks for replication at the receptor, post-entry, and viral encoding levels. Also, the potential for shedding of RCLs is very low, even when there are RCLs in vector preparations. Thus, mice act as a biological containment system for the vectors.

Animal husbandry and housing issues surrounding the use of lentiviral vectors can be separated into two events: the acute phase around inoculations; and the subsequent housing period. These guidelines only apply to the latest generation lentiviral vector systems with transgenes that pose low risk (e.g., GFP) as determined by the IBC, and immuno-competent mice.

- Inoculations- conduct under BSL2 conditions (ABSL2,BL2-N)
    - Transport inoculum to the animal procedure area within secondary containment
    - Inoculate mice in a biosafety cabinet. Wear appropriate personal protective equipment, including double gloves and protective gown. Consider anesthetizing animals and/or the use of puncture resistant gloves (e.g., vendors 1,2)
    - Use safer, engineered needles or needleless systems
    - Routinely decontaminate all work surfaces
    - Thoroughly cleanse and sanitize the inoculation site before placing the animal back in the cage
- Housing- the IBC and the IACUC will consider housing animals under BSL1 conditions (ABSL1,BL1-N) **if**:
    - Cages are maintained on ventilated racks or filter topped cages, at least for the immediate post-inoculation period
    - Inoculated mice are transferred, within a biosafety cabinet, to fresh cages no sooner than four days post-inoculation
    - Cages with old bedding are autoclaved and bedding is collected in red biohazard bags and incinerated

  Mice can then be maintained utilizing standard husbandry procedures and housing conditions (ABSL1, BSL1-N).

Inoculated animals must be closely monitored for signs of "not doing well" or signs of obvious illness. Appropriate actions for these animals will be determined by the CARE Veterinarians in consultation with the Principal Investigator.

Mice that undergo embryo transfer (i.e., embryos are transfected with lentiviral vectors in the laboratory and then surgically transplanted into the uterus) or are transplanted with lentiviral transfected cells in culture, with low risk transgenes, can be housed under standard BSL1 conditions and will not require any additional requirements. The four day post-inoculation precautions will not apply.

Safety testing of mice or embryos for generation of RCLs is not required at this time, although the IBC may encourage it in certain situations.

There may be circumstances where mice are not considered a containment system. For example, mice engrafted with human cells or mice lines that are permissive for HIV-1 replication (e.g., SCID mouse with human immune system). Because of the potential for replication of HIV-1, these inoculated animals must be maintained at higher containment (ABSL2, BSL2-N) for the duration of the experiment.

*** Prior to initiating any animal studies with lentiviral vectors, the investigators and animal care staff must meet with the Biosafety Officer and a CARE Veterinarian to assess procedures and review any potential concerns.

Applications in Other Animal Systems

Applications in other animal systems will be evaluated individually. Contact the IBC before contemplating and initiating any studies.

Non-Human Lentiviral Systems

Non-human lentiviral vectors, such as FIV (feline immunodeficiency virus) and EIAV (equine infectious anemia virus) are also used to permanently transduce cells. Although the viruses from which these vectors are derived are not human pathogens, the corresponding vector systems may contain heterologous envelope proteins (e.g., VSV-G). The VSV-G envelope protein, for example, extends the tropism of the vector so that these vectors can transduce human cells. Thus, the risks for personnel working with these vector systems are very similar to those for HIV-1 based vectors. Laboratory work with these non-human vectors must be conducted

under BSL2 conditions, identical to those listed above. Non-permissive mouse lines can be maintained at BSL1 (ABSL1, BSL1-N), given the same limitations for HIV-1 based vectors.

Procedures for Accidental Exposures

Accidental exposures to preparations of lentiviral vectors can include needlesticks, direct contact with cuts or other skin openings, and splashes to the mucous membranes. However, consequences of exposure and appropriate post-exposure treatments are not well defined, and so the emphasis should be placed on prevention. Nonetheless, if an exposure should occur, personnel should wash exposed areas for several minutes with soap and water. Eyewash or running water from a sink can be used to rinse mucous membranes. Exposed personnel should seek medical evaluation, preferably at the Occupational Medicine clinic at Gannett Health Services. Lastly, document all exposures in a Cornell University Injury and Illness Database.

References

1. Biosafety Considerations for Research with Lentiviral Vectors: Recombinant DNA Advisory Committee Guidance Document. National Institutes of Health, 2006.
2. NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules
3. Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition.