

Biosafety Considerations for Research with Lentiviral Vectors

Recombinant DNA Advisory Committee (RAC) Guidance Document

Background: The use of lentiviral vectors has been increasing because the vector system has attractive features; however, such research also raises biosafety issues. The NIH Office of Biotechnology Activities has received frequent questions regarding the appropriate containment for lentiviral vectors, particularly those derived from HIV-1. Because the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* do not explicitly address containment for research with lentiviral vectors, the RAC was asked to provide additional guidance for institutional biosafety committees (IBCs) and investigators on how to conduct a risk assessment for lentiviral vector research. At the March RAC 2006 meeting ([webcast](#)), the RAC offered the following findings and recommendations.

Risks of lentivirus vectors: The major risks to be considered for research with HIV-1 based 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.

General criteria for risk assessment of lentivirus vectors: Decisions about containment should take into account a range of parameters/considerations including:

- the nature of the vector system and the potential for regeneration of replication competent virus from the vector components,
- the nature of the transgene insert (e.g., 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 below*)

General containment considerations: Either BL2 containment or enhanced BL2 containment is often appropriate in the laboratory setting for research involving the use of advanced lentivirus vector systems that have multiple safety features and that segregate vector and packaging functions onto four or more plasmids. Enhanced BL2 containment may include in addition to attention to sharps (and use of safety needles where feasible), the use of personal protective equipment intended to reduce the potential for mucosal exposure to the vector. In most such research, these levels of containment are also expected to be appropriate even when producing large volumes of HIV-1 vectors (>10 L).

The appropriate containment level for specific lentivirus vector research is, of course, determined following a complete risk assessment and local IBC review. The following sections discuss some considerations which should form an important part of the biosafety assessment for research involving lentivirus vectors.

Potential for generation of replication competent lentivirus (RCL) from HIV-1 based lentivirus vectors: The potential for generation of RCL from HIV-1 based lentivirus vectors depends upon several parameters, the most important of which are

- the number of recombination events necessary to reassemble a replication competent virus genome and
- the number of essential genes that have been deleted from the vector/packaging system.

On this basis, later generation lentivirus vector systems are likely to provide for a greater margin of personal and public safety than earlier vectors, because

- they use 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 should also be considered in the overall safety assessment by the IBC),
- they separate vector and packaging functions onto four or more plasmids and
- they include additional safety features (e.g., they do not encode Tat, which is essential for replication of wild-type HIV-1).

In contrast, earlier vector systems (such as two-plasmid vector systems) may have a higher potential for generation of RCL.

RCL testing: The National Gene Vector Laboratory (NGVL) has produced over 60 liters of HIV-1 vector and has screened supernatant and cells from different vector systems, using different assays, without detecting RCL (K. Cornetta, personal communication of unpublished data). This suggests that the frequency of RCL generation using lentivirus vectors is very low. It may not, however, be zero. There is a need for continued investigation of RCL generation using lentivirus vectors, in order to inform and advance the field of lentivirus vector technology.

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.

Animal studies: Some animals, such as wild-type mice, cannot support replication of infectious HIV-1. As a result, the potential for shedding of RCL from such animals is very low (even if RCL were present in the original vector inoculum). IBCs may consider the biosafety issues associated with animal husbandry and housing *after* the initial injection separately from the initial inoculation itself. In general, the initial delivery of vector should be performed under Biosafety Level 2 for Animals (BL2-N) or under enhanced BL2-N containment (see "*General containment considerations*"), so as to minimize the risk of autoinoculation by the investigator. However, it may be permissible to reduce the containment level at some point following vector delivery. For example, if there is no expectation of infection (see below), the site of inoculation has been thoroughly cleansed, and the bedding changed, it may be acceptable to consider reducing containment from BL2-N to BL1-N within a few days (the specific time period can be specified by the local IBC, and may vary anywhere from 1-7 days depending on local and experimental considerations). 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 higher level of containment.

Other lentivirus vectors: Some non-human lentivirus vectors (e.g., FIV, SIV, EIAV, etc.) are also in use. Of these, the most frequently encountered are feline immunodeficiency virus (FIV) vectors. In the Appendix B-V of the *NIH Guidelines*, a containment level appropriate for Risk Group 1 agents is recommended for use of certain animal viral etiologic agents not associated with disease in healthy human adults. However, replication-defective vectors in which a heterologous envelope (such as VSV-G) is used for vector packaging may require BL2 containment in the laboratory setting, since

these vectors have the potential to transduce human cells, and thus have the potential to cause insertional mutagenesis. Under circumstances in which mice are not permissive hosts for FIV replication, BLN-1 containment may be acceptable for mouse housing and husbandry when dealing with mice that have received FIV vectors (subject to the considerations noted above).

Summary: A comprehensive risk assessment and determination of containment for research with lentiviral vectors should consider the nature of the vector system, transgene insert, and type of manipulations involved. For many experiments, either BL-2 or enhanced BL-2 will be appropriate. Examples of biosafety considerations and risk assessments for three different scenarios are included below.

Examples of Biosafety Considerations

Vector Considerations

- Potential for generation of RCL
 - Vector and packaging functions separated onto multiple plasmids
 - Deletion of viral genes
- Viral Env used in packaging system
 - Non-native Env (decrease potential for generation of RCL)
 - Coat protein that increases species or cell type tropism of parent virus (e.g., VSV-G)
- Safety modifications (e.g., no expression of Tat)

Transgene Considerations

- Oncogene
- Non-oncogene

Vector Generation Considerations

- Laboratory scale
- Large scale

Animal Research Considerations

- Permissive host
- Non-permissive host
- Animal engrafted with permissive cells
- Vector Administration (e.g., injection)
- Housing and husbandry

Practices, Containment Equipment and Training Considerations

- Training in use of PPE
- Availability of safety equipment (e.g., sealed centrifuge rotor cups)
- Laboratory-specific safety and spill cleanup protocols
- Availability of on-site occupational health support in the event of accident

Biosafety Considerations and Risk Levels

Biosafety Considerations

Higher Risk

Lower Risk



Vector Design

- Vector packaging functions on two plasmids
- Expression of viral genes
- Vector and packaging functions separated onto multiple plasmids
- Deletion of viral genes

Transgene

- Oncogene
- Non-oncogene

Vector Generation

- Large scale
- Laboratory scale

Animal Hosts

- Permissive host
- Non-permissive host
- Animals engrafted with human cells

Animal Manipulation

- Vector administration (e.g., use of sharps during injection)
- Housing and husbandry (no use of sharps)

EXAMPLE SCENARIOS

EXAMPLE ONE: *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?* A = LOW (100 ml)
2. *What is the nature of the vector?* A = 4-Plasmid System
3. *What is the nature of the insert?* A = Non-Oncogenic

Tentative Safety Assessment = BL2

(Note that the use of primary human cells would require BSL2 containment, independent of the vector, as well as use of Universal Precautions and compliance with the OSHA standard for Bloodborne Pathogens)

EXAMPLE TWO: *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 vector to be produced?* A = LOW (100 ml)
2. *What is the nature of the vector?* A = 2-Plasmid System (non-commercial)
3. *What is the nature of the insert?* A = Non-Oncogenic

Tentative Safety Assessment = BL2 enhanced

BSL2 "enhanced" stipulations might include:

- Avoidance of needles and sharps, where possible
- Use of a containment hood for all work with the vector (including the loading and unloading of centrifuge rotors, which should have an aerosol-tight seal)
- Use of personal protective equipment [PPE] designed to prevent a mucosal exposure/splash to the face and exposure of hands (especially in persons with broken skin or open cuts)
- A requirement for an in-person consultation between biosafety staff and lab personnel prior to initiation of experiments

EXAMPLE THREE: *In vivo* study A

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

Considerations

1. *What is the amount of vector to be produced?* A = LOW (100 ml)
2. *What is the nature of the vector?* A = 4-Plasmid System
3. *What is the nature of the insert?* A = Non-Oncogenic (*: see below)
4. *What is the nature of the animal host?* A = Non-permissive for HIV-1

Tentative Safety Assessment = BL2-N for lab work and initial injection of mice (which would probably be done using a stereotactic frame); after 1-7 days, animals could be moved to BL1-N containment.

Added explanation:

- 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 increased biocontainment.
- Stereotactic injection frames cannot easily be placed into a laminar flow hood, and may use a syringe or pulled glass pipette for inoculation; they may also use a pump to ensure a slow rate of delivery of the agent. BL-2 containment does NOT require the use of a biosafety cabinet, and is therefore compatible with the use of a stereotactic frame, even if that frame is not contained within a laminar flow cabinet.

Additional points to consider:

- An in-person consultation between biosafety staff and lab personnel prior to initiation of experiments may be a useful stipulation
- One might also impose additional biosafety enhancements during the injection process, perhaps by requiring use of additional PPE above and beyond the stipulated requirements associated with BL2/BL2-N. See Example 2 for examples of such stipulations.