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 BL2 or BL2-N, according to the animal model, or under enhanced BL-2/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/BL2-N to BL1/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, BL1 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 • Animals engrafted with human cells	• Non-permissive host
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 BL2 containment, independent of the vector, as well as use of Universal Precautions and compliance with the OSHA standard for Bloodborne Pathogens -- 29 CFR 1910.1030)

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

BL2 "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 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 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. BL2 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.

APPENDIX

Sections from the *NIH Guidelines*

General Considerations

Section II-A. Risk Assessment
Section II-A-3. Comprehensive Risk Assessment. BL2 containment is recommended for activities involving all blood-contaminated clinical specimens, body fluids, and tissues from all humans, or from HIV – or HBV-infected or inoculated laboratory animals. Activities such as the production of research-laboratory scale quantities of HIV or other bloodborne pathogens, manipulating concentrated virus preparations, or conducting procedures that may produce droplets or aerosols, are performed in a BL2 facility using the additional practices and containment equipment recommended for BL3. Activities involving industrial scale volumes or preparations of concentrated HIV are conducted in a BL3 facility, or BL3 Large Scale if appropriate, using BL3 practices and containment equipment. Appendix

Section III-D. Experiments That Require Institutional Biosafety Committee Approval Before Initiation
Section III-D-3. Experiments Involving the Use of Infectious DNA or RNA Viruses or Defective DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems. Recombinant DNA or RNA molecules derived therefrom, which contain less than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family (see Section V-J, *Footnotes and References of Sections I-IV*) being considered identical (see Section V-K, *Footnotes and References of Sections I-IV*), are considered defective and may be used in the absence of helper under the conditions specified in Section III-E-1, *Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus*.

Section III-E. Experiments That Require Institutional Biosafety Committee Notice Simultaneous with Initiation
Section III-E-1. Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus. Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family being considered identical [see Section V-J, *Footnotes and References of Sections I-IV*] may be propagated and maintained in cells in tissue culture using BL1 containment. For such experiments, it must be demonstrated that the cells lack helper virus for the specific Families of defective viruses being used. If helper virus is present, procedures specified under Section III-D-3, *Experiments Involving the Use of Infectious Animal or Plant DNA or RNA Viruses or Defective Animal or Plant DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems*, should be used. The DNA may contain fragments of the genome of viruses from more than one Family but each fragment shall be less than two-thirds of a genome.

Animal Studies

Section III-D-4-a. Recombinant DNA, or DNA or RNA molecules derived therefrom, from any source except for greater than two-thirds of eukaryotic viral genome may be transferred to any non-human vertebrate or any invertebrate organism and propagated under conditions of physical containment

comparable to BL1 or BL1-N and appropriate to the organism under study (see Section V-B, *Footnotes and References of Sections I-IV*). Animals that contain sequences from viral vectors, which do not lead to transmissible infection either directly or indirectly as a result of complementation or recombination in animals, may be propagated under conditions of physical containment comparable to BL1 or BL1-N and appropriate to the organism under study. Experiments involving the introduction of other sequences from eukaryotic viral genomes into animals are covered under Section III-D-4-b, *Experiments Involving Whole Animals*. For experiments involving recombinant DNA-modified Risk Groups 2, 3, 4, or restricted organisms, see Sections V-A, V-G, and V-L, *Footnotes and References of Sections I-IV*. It is important that the investigator demonstrate that the fraction of the viral genome being utilized does not lead to productive infection. A U.S. Department of Agriculture permit is required for work with plant or animal pathogens (see Section V-G, *Footnotes and References of Sections I-IV*).

APPENDIX B. CLASSIFICATION OF HUMAN ETIOLOGIC AGENTS ON THE BASIS OF HAZARD

Appendix B-III-D. Risk Group 3 (RG3) - Viruses and Prions

Retroviruses
--Human immunodeficiency virus (HIV) types 1 and 2

BL2 Facilities

Appendix G-II-B-3. Containment Equipment (BL2)
Appendix G-II-B-3-a. Biological safety cabinets (Class I or II) (see Appendix G-III-L, *Footnotes and References of Appendix G*) or other appropriate personal protective or physical containment devices are used whenever:

Appendix G-II-B-3-a-(1). Procedures with a high potential for creating aerosols are conducted (see Appendix G-III-O, *Footnotes and References of Appendix G*). These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of materials whose internal pressures may be different from ambient pressures, intranasal inoculation of animals, and harvesting infected tissues from animals or eggs.
Appendix G-II-B-3-a-(2). High concentrations or large volumes of organisms containing recombinant DNA molecules are used. Such materials may be centrifuged in the open laboratory if sealed beads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

Appendix G-II-B-4. Laboratory Facilities (BL2)
Appendix G-II-B-4-a. The laboratory is designed so that it can be easily cleaned.
Appendix G-II-B-4-b. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
Appendix G-II-B-4-c. Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.
Appendix G-II-B-4-d. Each laboratory contains a sink for hand washing.
Appendix G-II-B-4-e. If the laboratory has windows that open, they are fitted with fly screens.
Appendix G-II-B-4-f. An autoclave for decontaminating laboratory wastes is available.