Thoughts on Lentivirus Vector Discussion Questions
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What factors should be considered in a biosafety assessment of lentiviral vector research with regard to

a. The parental virus the vector is derived from (e.g., HIV-1, FIV, EIAV, etc.)

The nature of the genes and sequences remaining from the parental vector (and the potential risk of recombination) is of greater importance than the virus from which the vector was derived. This statement assumes that all are packaged using pseudotyping. FIV and EIAV are as far as we know are incapable of establishing infection in humans and not known to pose a risk of recombination with lentiviruses known to infect humans. Nonetheless, should mixed infection occur, it would be theoretically by possible for such recombinations to occur.

b. Safety modifications to the vector system (e.g., deletion of viral genes, sequence overlap creating potential for recombination, SIN vector, etc.)

Vectors retaining as little viral sequence as possible and no viral genes are ideal. Retention of any HIV genes, particularly the env gene, should be avoided. (Necessary genes for packaging should be supplied on other plasmids, see below) SIN vectors do not necessarily eliminate the potential for recombination and thus while they do provide additional benefits with regard to replication of the vector, they may not actually provide significant additional benefit in circumstances where there is the potential presence of other replicating lentiviruses.

c. The system used to generate vector (e.g., 2, 3, or 4 plasmid transient transfections, stable packaging cell lines, etc.)

Use of packaging systems in which a minimum of three plasmids are used, one to provide the envelop (VSV-G), one for the vector and one to provide retrovirus genes required for packaging (the minimum needed being gag-pol and rev)should always be used. It would be much better to provide gag-pol on one plasmid (these probably cannot be easily separated) and rev on another, making a system involving 4 plasmids. Other lentivirus genes should not be required for most applications. Transfections using multiple plasmids are easily possible and it is difficult to see why a 4 plasmid system cannot be the standard for most experiments.

d. Pseudotyping (e.g., VSV-G etc.)

For most applications, there should be no need to use a lentivirus vector expressing a lentivirus envelop gene. This statement is particularly true for HIV-based vectors. Vector preparations packaged with HIV env should be avoided.

What factors should be considered in determining containment for different types of manipulations of lentiviral vectors such as

a. Vector generation

The presence of virus sequences and virus genes from the lentivirus is a consideration as a possible source of contamination. It is particularly important that care be taken to insure that vector plasmid preparations are not contaminated with lentivirus gene sequences.

b. Tissue culture

The presence of cells infected with lentiviruses that might serve as a possible source of replication competent virus or provide viral sequences that would be able to recombine with the vector is a consideration.

c. Small and large animal work

a. Vector administration

There are no special containmant issues that would make vector administration different from other circumstances described above. That said, it is extremely likely that the use of sharps will be involved in administration. In general use of sharps should be avoided when dealing with these preparations to minimize the risk to the experimentalist. Thus, increased care and instruction in conducting the procedure needs to be in place.

b. Housing

Once administration and cleansing of the site is completed, it seems reasonable to house most animals under BL1 conditions. In reality, it will be difficult to use such vectors in rodents that must be housed under higher levels of containment. Assuming that the animals contain no human cells and contain no lentivirus (HIV) sequences, such levels should be sufficient.

d. Animals permissive for viral replication

Animals permissive for virus replication should be housed under the same conditions mandated for these animals inoculated with live virus.

e. Large scale production

Risk of contamination of the preparation with other sequences, including those that may have been prepared earlier in the facility, must be taken into consideration.

What consideration should be given to the type of transgene expressed from the vector?

The presence of transgenes that could have disease causing potential (oncogenes, etc) should be avoided.

When should testing for replication competent lentivirus (RCL) be considered?

In most experimental situations where these vectors are used, it does not seem necessary to perform rigorous testing for RCL. The risks are extremely small and the tests that might be employed are usually not standard in the laboratories that use these vectors. Routine testing when vectors are packaged with VSV-G and used in situations where no lentiviruses genes are present is not required in my view.

What RCL assays may be useful?

In circumstances where there could be the potential for recombination, perhaps an assay such as the PERT assay (product-enhanced reverse transciptase assay) or assay to detect viral gag might be considered.

For research in which enhanced BL2 containment is considered appropriate, what types of enhancements to practices would be most useful?

For most work, it would seem that BL2 containment is sufficient for these vectors. This statement assumes that contact with HIV-infected individuals is not at issue, a factor that cannot be completely controlled. Enhancements such as air handling controls are not necessary in my view. In addition, RCL testing in most cases (see above) should not be a requirement for BL2 level status. However, mandating that all work be conducted in biosafety cabinets, minimizing contact with skin, eyes, mucous membranes and restricting the use of any sharps are appropriate enhancements that should be in place.