RAC Discussion of Lentivirus Containment

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Risk assessment and containment determination for research with lentiviral vectors.

Background: OBA has been receiving frequent questions regarding appropriate containment for lentivirus vectors. The NIH guidelines don't address lentiviral vectors very explicitly, but one can interpret the Guidelines to permit vector containment under the same standards that might be applied to infectious HIV-1 (i.e., BL3 practices in a BL2 facility), with potential for lowering biocontainment based on results from RCL testing. However, some IBCs and investigators, particularly those using the Invitrogen kit, are recommending BL2 containment for vector generation and subsequent research without RCL testing. Hence,there is a need for some general guidance or "points to consider" when using lentivirus vectors in the preclinical setting.

Invitrogen Virapower system: An example of a 3rd generation lentivirus vector. The Virapower system, like other non-commercial systems, is a "third-generation" vector system that incorporates several safety features (http://www.fda.gov/ohrms/dockets/ac/01/slides/3794s2_02_takefman/sld016.htm). These include:

- Only 3 viral genes are used in vector system (gag, pol on one plasmid, and rev on another). Tat is not expressed in the system.
- HIV-1 Env is replaced by the VSV-G gene
- The genes encoding structural and other essential genes are separated onto 4 plasmids
- The vector is "self-inactivating" due to a deletion in the 3′ LTR (ΔU3)

The current vector manual for Invitrogen's Virapower system (http://www.invitrogen.com/content.cfm?pageid=10418) recommends that users "*treat lentiviral stocks generated using this System as Biosafety Level 2 (BL-2) organisms and strictly follow all published BL-2 guidelines with proper waste decontamination"* and exhorts users to *" exercise extra caution when creating lentivirus carrying potential harmful or toxic genes (e.g. activated oncogenes)*." The manual also notes that "*since safety requirements for use and handling of lentiviruses may vary at individual institutions, we recommend consulting the health and safety guidelines and/or officers at your institution prior to use of the ViraPower™ Lentiviral Expression System*."

Potential criteria for risk assessment of lentivirus vectors

Based on the NIH Guidelines, it may be appropriate to consider BL2+ containment (BL3 practises in a BL2 facility) as a starting point for containment of HIV-1 based lentivirus vectors. The Guidelines allow for local modification of containment levels, and the potential for lowering biocontainment may depend upon a range of parameters/considerations such as:

(i) negative RCL testing (*see note below*),
(ii) the nature of the vector system (e.g., what is the potential for regeneration of infectious HIV-1 from the vector components? Is the vector a 1-, 2-, 3- or 4- plasmid system? What genes are deleted from the vector/packaging system? Is the vector "self-inactivating"?)
(iii) the nature of the insert (e.g., known oncogenes or genes with high oncogenic potential may merit special care)
(iv) the vector titer,

(v) the inherent biological containment of the animal host, if one is conducting *in vivo* studies (e.g., HIV-1 does not replicate in wild-type mice but it may replicate in mice that have been engrafted with human immune cells or human T cell lines) (*see note below*)

(vi) any other relevant issues

Personal thoughts on points (i) and (v):

(i) RCL testing: I am not compelled by the utility of RCL testing data, in many cases. This is because there is no reliable assurance of assay/data standardization between different basic science labs (most of which have little or no experience with infectious HIV-1, its propagation and p24 assays). RCL testing is also of debatable value when using vector systems that cannot possibly result in the generation of infectious HIV-1 (such as systems in which the lentivirus vectos/packaging system lacks HIV-1 Env and uses a fully heterologous coat protein such as VSV-G to wrap the vector core).

(v) Mouse studies: Mouse studies are a complex issue. HIV-1 transgenic mice should be considered on a case-by-case basis, and (if they contain an intact HIV-1 provirus) may require A high level of containment. However, studies using lentivirus vectors in mice involve the direct inoculation of vector particles into a live animal via a needle or other injection device. The issue of animal (mouse) husbandry and housing AFTER the initial injection is worth considering, separately from the initial inoculation itself. In general, the initial delivery of vector should be performed under ABL2/ABL2+ biocontainment (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 (after thorough cleansing of the site of inoculation) – at least in wild-type mice that have not been engrafted with human cells. The reality is that ABL2 housing space for small animals is limited at many institutions, and may not be necessary for all experiments using lentivirus vectors in mice. *It is should be noted that the NIH Guidelines permit considerable flexibility when using vectors that are <2/3 genome size, such as many of the second- and third-generation lentivirus vectors.*

FIV vectors: Finally, some non-human lentivirus vectors are in use. Of these, the most frequently encountered are feline immunodeficiency virus (FIV) vectors. FIV vectors are based on a virus that is assessed at BL1 in the NIH Guidelines. However, replication-defective FIV 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 a risk for insertional mutagenesis. Since mice are not permissive hosts for FIV replication (under normal circumstances), ABL1 containment may be acceptable for mouse housing & husbandry when dealing with mice that have received FIV vectors (subject to the considerations noted above).