

Viral Vectors and Biological Safety

Viral vectors are often designed so that they can enter human cells and deliver genes of interest. Viral vectors are usually replication-deficient – genes necessary for replication of the virus are removed from the vector and supplied separately through plasmids, helper virus, or packaging cell lines.

There are several biosafety concerns that arise with the use of viral vectors including:

- 1) Tropism (host range) – viral vectors that can enter (infect) human cells are often used.
- 2) Replication-deficient viral vectors can gain back the deleted genes required for replication (become replication-competent) through recombination – referred to as replication-competent virus (RCV) breakthroughs.
- 3) Genes may be expressed in tissues and/or organisms where they are normally not expressed. In the case of some genes such as oncogenes, this could have far-reaching negative consequences.

When evaluating safety for use of viral vectors, a number of factors need to be considered including: Risk Group (RG) of the organism; tropism (organism and tissue); route of transmission; whether the virus integrates into the host genome; and the specific gene(s) being introduced. Please contact the Office of Biological Safety (OBS) for more information on physical barriers and safety practices to use with specific viral vectors. This article concentrates on biological barriers that can be employed to improve safety when using viral vectors.

Viral vectors frequently used are:

- Retrovirus/lentivirus
- Adenovirus
- Adeno-associated virus (AAV)
- Poxvirus
- Herpes virus
- Alphavirus
- Baculovirus

Amphotropic murine leukemia virus (MLV) – also called Moloney murine leukemia virus (MMLV) – and adenovirus are common viral vectors used to introduce genes into human cells. **Amphotropic** means the virus is able to infect human cells – this is in contrast to an **ecotropic** MLV that can only infect murine cells.

Both amphotropic MLV and adenovirus are Risk Group 2 (RG2) pathogens. Both these viral vectors infect human cells and both have potential for RCV breakthroughs. Amphotropic MLV integrates into the host's genome – this translates into stable expression of introduced genes and the potential for insertional mutagenesis of host genes. Adenoviruses are cold germs that can be transmitted by aerosol and can cause eye damage. Adenoviral vectors are known to have a relatively high rate of RCV breakthroughs compared to other viral vectors.

There are a number of ways to improve safety when working with viral vectors. Briefly these include:

- 1) Consider the alternative of nonviral vectors. Advances in nonviral vector technology may mean that a nonviral vector will serve your purpose.
- 2) Limit tropism – narrow host range and/or tissue infected. Even if you need your vector to infect human cells, you may be able to engineer your viral vector so that it only infects specific tissue(s).
- 3) Use strategies to decrease chances of RCV breakthroughs.
 - Split genomes of viral replication genes. Having replication genes on different constructs means that more recombination events would need to occur in order to get a RCV breakthrough.
 - Remove viral regulatory regions. This decreases the chance of homologous recombination occurring.
 - Produce virus as a transient single batch (simultaneous transfection of plasmids) rather than as continuous culture (use of a packaging cell line with replication genes integrated into the genome of the cell line). There is an increased risk of RCV breakthroughs with the use of packaging cell lines, especially with large-scale production.
- 4) For viruses with complex genomes like adenovirus, delete as much of the viral coding sequence in the vector as possible (“gutless” adenoviral vectors). This helps to eliminate expression of viral genes in transduced cells.
- 5) Use ecotropic MLV with methods that allow the virus to enter human cells in a limited manner. This strategy would be especially relevant for introduction of genes such as oncogenes, mutant tumor suppressor genes, mutant repair genes and some signal transduction pathway genes.
 - Method 1: First transfect the cell line with the ecotropic MLV receptor, and then infect that cell line with ecotropic MLV. The virus would not be able to infect any human cells that had not already been transfected with the ecotropic MLV receptor.
 - Method 2: Use ecotropic MLV linked to poly-lysine (retrovirus molecular conjugate). The ecotropic virus can enter human cells in this form but any RCV breakthrough virus that arise would not be linked to poly-lysine and therefore would not be able to infect human cells.
- 6) Use controlled recombination (site-specific recombination mechanisms) to activate/inactivate viral replication genes.

When using viral vectors, ways to optimize safety must be considered. Training staff to safely handle viral vectors (and animals infected with viral vectors) is the responsibility of the Principal Investigator. OBS would like to assist in improving safety whenever possible.

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