

## How to Complete a Risk Assessment of Retroviral Experiments (other than Lentivirus) in Animals and Cell Culture

**1. Organism or Agent:** RETROVIRAL VECTORS

**2. Synonym:** MoMLV, X-MLV, P-MLV, A-MLV, GALV, HERV-w, SIV-1, FIV-1

MoMLV, Moloney Murine Leukemia Virus; X-MLV, xenotropic MLV; P-MLV, polytropic MLV; A-MLV, amphotropic MLV; GALV, gibbon ape Leukemia Virus; HERV-W, human endogenous retrovirus group W; SRV 1-5, simian retroviruses type 1-5; FIV, feline immunodeficiency virus;

- 3. Characteristics:** Single stranded RNA virus, enveloped icosahedral nucleocapsid, glycoprotein envelope, reverse transcriptase
- 4. Containment Requirements:** Depends on agent: Usually biosafety level 2 practices, containment equipment and facilities for all activities involving the manipulation of the virus; primary containment devices and biological safety cabinets are recommended. Centrifuge safety precautions, secondary containers for transport between incubator and BSC. Keep hands away from the eyes, nose and mouth in order to avoid potential exposure of the mucous membranes; eye goggles or face shields may assist in accomplishing this objective.
- 5. Manipulations of Retroviral Vectors:** Depending on the vector, work may need to be performed within a biosafety cabinet, and the use of sharps including needles, blades and glassware should be minimized.
- 6. Spills:** Allow aerosols to settle; wear protective clothing including an N95 respirator, gently cover spill with paper towel and apply disinfectant, starting at perimeter and working towards the center; allow sufficient contact time before clean-up (30 min).
- 7. Biohazardous Waste:** Collect in double red bags and transport in a rigid container.
- 8. Approved Disinfectants:**
- i. 0.05% Sodium Hypochlorite (1:10 bleach/water) allow 10 minutes of contact time.
  - ii Stabilized prediluted bleach (preferred to lab prepared bleach)
  - . Other HIV-approved hospital disinfectants
  - iii. Alcohols are not acceptable disinfectants.
- 9. Disposal:** Decontaminate before disposal; steam sterilization, incineration, chemical disinfection.
- 10. Storage:** Store in sealed containers appropriately labeled with a biohazard label, description and contact information.
- 11. Pathogenicity:** Hazards depend on multiple factors: actively dividing cell lines (state pseudotyping, as VSV-G increases risk to non-dividing cells), whether the vector is capable of infecting human cells (state tropism), whether the vector is replication competent (risk of recombination events), insertional mutagenesis (increasing cancer risk), and the specific transgenes present in the vector.
- 12. Modes of Transmission:** Virus may be transmitted in the following ways: 1) a skin puncture or injection, 2) ingestion, 3) contact with mucous membranes (eyes, nose,

or mouth), 4) contact with non-intact skin, and 5) low risk exposures include bites from an animal, percutaneous contact with body fluids from an animal and aerosols.

Retrovirus	Genus	Receptor	Type	Function	Tropism
MoMLV	Gammaretrovirus	CAT-1	TM14	Amino acid transport	Ecotropic, mouse
X-MLV	Gammaretrovirus	XPR1	TM8	Unknown	Xenotropic, human, other
P-MLV	Gammaretrovirus	XPR1	TM8	Unknown	Polytropic, mouse & human
A-MLV	Gammaretrovirus	Pit-2	TM10-13	Phosphate Transport	Amphotrophic, mouse and human
GALV	Gammaretrovirus	Pit-1	TM10-13	Phosphate Transport	Primate, humans
HERV-W	Gammaretrovirus	RDR	TM9-10	Amino acid transport	Human
SRV-1 to 5	Gammaretrovirus	RDR	TM9-10	Amino acid transport	Primate
SIV-1	Lentivirus	CD4, CCR5, others	TM1, TM7	MHCII binding, chemokine receptor	Primate, human
FIV-1	Lentivirus	CXCR4, HS	TM7	Chemokine receptor	Feline, human

- 13. Length of gene expression:** Variable, may be months to years. Also long-term issues of insertional mutagenesis.
- 14. Communicability:** Replication incompetent vectors: Not communicable unless reversion to replication competence.
- 15. Medical surveillance and clinical treatment procedure:** No medical surveillance is required. Clinical Operating Procedure “Retroviral Vectors” must be listed on risk assessment.
- 16. Safety Generation of Vector:** State which generation vector is being used. Information can be found in manufacture’s documentation.
- 17. Stability in Environment:** At 37°C, the half-lives of MLV vectors were 4.5 +/- 1.0 hour (Virology 280, 124-131 (2001)).
- 18. Vector concentration, dosage per experiment:** State your stock vector concentration, and the amount used per experiment or kg.
- 19. Vector shedding from humans & animals:** In 10 out of 16 publications on in vivo retroviral gene therapy, the presence of vector in blood (primarily PBMCs) was demonstrated by PCR after intratumoral gene therapy for brain tumours [8,14,16,17,21,27], melanoma [3,7], and breast tumours [3], and intraperitoneal gene therapy for ovarian cancer [25,26]. Specifically, in 6 out of 8 publications describing gene therapy for brain tumours by intratumoral administration, vector sequences were found in PBMCs [8,14,16,17,21,27]. The duration of shedding varied from 1 to 28 days after administration. In none of these publications was the finding of vector

genomic material confirmed as infectious viral particles (see J Gene Med 2007; 9: 910–921 for all reference numbers). Animals dosed intravenously may shed virus for up to 72 hours; animals dosed intracranially may shed for up to 24 hours.

**20. Transgene Information:** Discuss effects of transgene on animal or cell line. A good source for understanding the transgene being silenced or over-expressed is GENE CARDS (<http://www.genecards.org/>). A snapshot of a sample gene card is shown below:

The screenshot shows the GeneCards website interface. At the top, there are logos for GeneCards, Wellcome Institute of Genomics, and the Wellcome Trust. Below the logos, there is a navigation menu with links like 'Home', 'GeneCards Guide', 'Suite', 'Terms and Conditions', 'About Us', 'User Feedback', and 'Mirror sites'. A search bar is present with the text 'Set Analyses: [dropdown] [dropdown] keyword(s) Search Advanced Search'. The main content area is titled 'TNFRSF10B Gene protein-coding GPC: 73' and 'Tumor Necrosis Factor Receptor Superfamily, Member 10b'. It includes a 'Jump to Section...' dropdown, a list of aliases, and a table of related diseases. The table lists diseases such as 'Mycosis fungoides', 'Lymphoma', and 'Malignant melanoma'. External IDs and a list of previous GeneCards identifiers are also provided.

To better understand potential human outcomes from accidental silencing, you can see if information exists in the Mouse Genome Informatics (<http://informatics.jax.org/>)

You must discuss the potential effects due to accidental worker exposure. If unknown, state that. Is the gene sequence or siRNA specific to an animal, humans or could it affect both.

**Animal Biosafety and Containment:** Following transfection, animals are held at ABSL-2 for 72 hours. Following a cage change (performed at BSL-2), the animals may be housed at ABSL-1.