University of Pittsburgh Institutional Biosafety Committee
Guidance on Biosafety Level Assignment for Murine Retroviral Vectors

Approved July 13, 2015

Background

The use of murine-derived retroviral vector systems is becoming commonplace with the advent of commercially-available expression systems that have increased efficiency of gene expression with reduced biosafety concerns. The integration of the provirus into the cellular genome is passed on to the next generation of cells, and provides means to achieve long-term gene expression.

Murine retroviruses that can only infect rodent cells are termed ecotropic and thus are not able to infect human cells unless specifically engineered to do so. Amphotropic viruses, such as VSV-g pseudotyped viruses, are able to infect many cell types, including human cells. In general, the commercially available expression systems have been engineered to reduce the possibility of homologous recombination events, using such features as self-inactivation, so that although the viruses are able to infect cells (i.e., enter cells), they are physically unable to replicate in or produce infectious particles from the cells (replication-deficient, replication-defective, or replication-incompetent).

Sub-committee discussion on practices at other institutions

With respect to practices at other institutions, there seems to be a consensus for routinely assigning a higher level of safety practices in regard to animal waste handling and the specific animal holding facilities, for work with either ecotropic or amphotropic murine virus vector-based protocols.

Amphotropic or replication-competent murine retroviruses will require BSL-2/ABSL-2 containment and handling practices.

Other institutions, including Yale University, allow ecotropic murine virus systems using oncogenic transgenes to be approved at "ABSL-1+" with the exception of wearing face shields and using safety-engineered sharps devices; this is comparable to current practices in the University of Pittsburgh's DLAR ABSL-1 for animal containment. As discussed at the June 2015 IBC meeting, the animal housing definition of "ABSL-1" is not standardized, but left to institutional interpretation.

Murine cells transduced with ecotropic murine retrovirus produced in human cells or express toxins, oncogenes, or other genes with oncogenic activity, must be administered *in vivo* using ABSL-2 practices, but can be placed into ABSL-1+ housing post-administration.

The group noted that in addition to biosafety issues with animal use, there are animal biosecurity concerns for the Division of Laboratory Animal Resources (DLAR) to consider when assigning appropriate housing areas. The issue of resource space is under the jurisdiction of the DLAR, so that is not part of the IBC review or approval process.

Recommendation

BSL-1/ABSL-1+: Specific requirements for use of Murine Retroviral Vectors

The IBC will consider designating murine retroviral vectors for use at BSL-1/ABSL-1+ if the following criteria are met:

1. Viral vector must be a **replication-deficient, ecotropic** murine retroviral vector (Eco-MRV).
2. Transgene does not express an oncogenic protein or toxin.

Eco-MRVs described above, and/or non-human cell lines transduced *in vitro* with these vectors, may be administered to animals and animals may be housed at ABSL-1+.

BSL-2 in vitro/ABSL-1+ in vivo: Specific requirements for use of Murine Retroviral Vectors

The IBC will consider designating murine retroviral vectors for use at BSL-2 *in vitro*/ABSL-1+ *in vivo* if the following criteria are met:

1. Viral vector must be a **replication-deficient, ecotropic** murine retroviral vector (Eco-MRV).
2. Transgene expresses an oncogenic protein or toxin.

Eco-MRVs described above, and/or non-human cell lines transduced *in vitro* with these vectors, may be administered to animals using ABSL-2 practices at the time of injection and animals may be housed at ABSL-1+ immediately post-administration recovery.

BSL-2/ABSL-2: Specific requirements for use of Murine Retroviral Vectors

Murine retroviral vectors ***must*** be used at BSL-2/ABSL-2 if:

1. Viral vector is a replication-competent, ecotropic murine retroviral vector due to animal colony biosecurity implications.
2. Viral vector is a replication-deficient **amphotropic** murine retroviral vector.
3. Viral vector is used to transduce human and/or non-human primate cell and cell lines.

The table below summarizes the Institutional Biosafety Committee's recommendations for biosafety levels for work with murine retroviral vectors:

Summary of biosafety level requirements for use of murine retroviral vectors			
Viral Vector	**Use *in vitro***	**Use *in vivo***	
		Without Human Cells	**With Human Cells**
Murine Retrovirus – Ecotropic	BSL-1	♦ABSL-1+	ABSL-2
Murine Retrovirus – Ecotropic: expressing toxin, oncogene, or gene with oncogenic activity	BSL-2	♦ABSL-1+	ABSL-2
Murine Retrovirus – Replication-competent Ecotropic *OR* Amphotropic	BSL-2	ABSL-2	ABSL-2

♦ **Definition of ABSL-1+ at the University of Pittsburgh**

At the University of Pittsburgh, there is a specialized biosafety level defined as ABSL-1+. In ABSL-1+ facilities, additional work practices and containment equipment described in biosafety guidelines for work at ABSL-2 are used in an ABSL-1 facility:

1. Research personnel should use a biosafety cabinet when administering Eco-MRV or transduced cells to animals.
2. In addition to standard ABSL-1 personal protective equipment (e.g. disposable laboratory gown or coverall suit, shoe covers, gloves, hair bonnet), face protection (e.g. a face shield or combination of a surgical mask and safety glasses) must be worn by personnel administering Eco-MRV or transduced cells to animals.
3. Safety engineered sharps devices must be used when administering Eco-MRV or transduced cells to animals.
4. Cage cards must be labeled with the name of the agent and a crossed-out biohazard sticker to identify cages for specialized waste handling by DLAR personnel.
5. DLAR personnel shall collect bedding from specially-marked cages and dispose via the incineration only waste stream.
6. IBC requirements for use of Eco-MRV or transduced cells shall be described in the investigator's IBC approval letter and the EH&S risk assessment issued for approved IACUC protocols involving use of these agents. An implementation meeting will be required between the investigator and DLAR personnel prior to beginning work with Eco-MRV or transduced cells in animals.