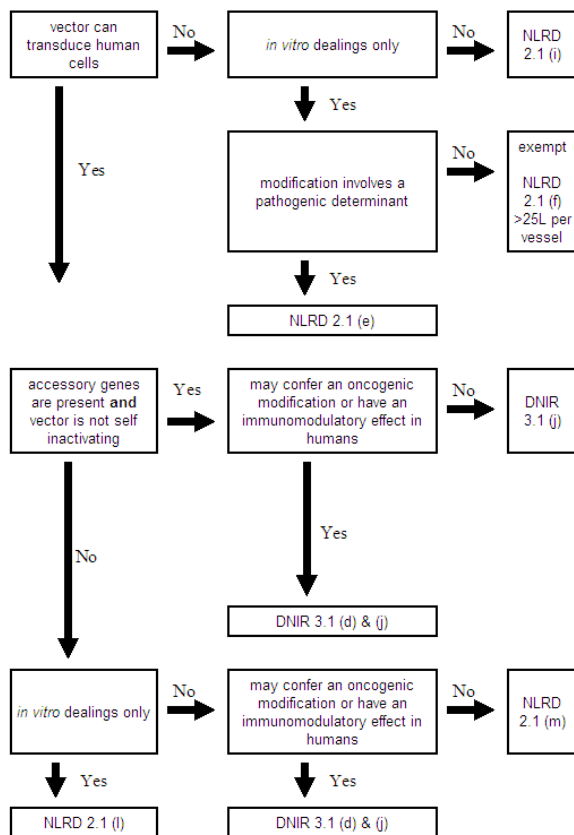# Gene Technology classification of dealings involving self-inactivating, replication defective, retroviral vectors.

The use of replication defective, retroviral vectors for research purposes has become increasingly popular due to their ability to stably integrate into chromosomes of target cells, the long term expression of proteins of interest, they do not transfer viral genes leading to cell destruction, their large cloning capacity and they transduce non dividing cells.

Under the Gene Technology regulations the classification of work involving the use of replication defective, retroviral vectors in tissue culture and *in vivo* depends on the DNA insert subcloned into the vector backbone and their ability to transduce human cells.

The OGTR provide the following flow chart to assist in classify dealings involving the use of replication defective retroviruses.

```
vector can            No    in vitro dealings only    No    NLRD
transduce human      ──►                             ──►   2.1 (i)
cells                                                        
  │                          │ Yes
  │ Yes                      ▼
  │                   modification involves a      No    exempt
  │                   pathogenic determinant      ──►   NLRD
  │                                                      2.1 (f)
  │                          │ Yes                      >25L per
  ▼                          ▼                          vessel
                      NLRD 2.1 (e)


accessory genes      Yes   may confer an oncogenic   No    DNIR
are present and      ──►   modification or have an   ──►  3.1 (j)
vector is not self         immunomodulatory effect in
inactivating               humans
  │                          │ Yes
  │ No                       ▼
  │                   DNIR 3.1 (d) & (j)
  ▼

in vitro dealings only  No  may confer an oncogenic   No   NLRD
                       ──►  modification or have an   ──►  2.1 (m)
  │                         immunomodulatory effect in
  │ Yes                     humans
  ▼                          │ Yes
NLRD 2.1 (l)                 ▼
                      DNIR 3.1 (d) & (j)
```

http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/viralvecchart2011-2-toc~viralvecchart2011_2-flow3

One of the benefits of using these retroviral vector systems is that they integrate into the chromosome of the target cell and therefore, after a period of time, the resulting transfected cell line or the animal will no longer contain virus but will continue to express the gene of interest. The safety features incorporated into replication defective, retroviral vectors prevent the virus from remobilising. Therefore, stably transfected cells lines or animals infected with replication defective, retroviral vectors may be reclassified to a lower level of dealing (and subsequently containment) after a period of time which is sufficient to ensure that infectious viral particles are no longer present.

In the case of transduced cell lines, after the virus is no longer active, dealings can be considered exempt dealings. In the case of animals, the dealing can be considered as either an exempt dealing for wild type animals or PC1-NLRD if the animals are transgenic or knockout mice, rats, rabbits or guinea pigs.

The OGTR do not provide a specified timeframe between viral transduction and reclassification of the work but instead leave it to the Institutional Biosafety Committee (IBC) to satisfy itself that no infectious virus remains.

After considering the literature on virus stability and clearance rates (**Appendix A**) the Monash University IBC has concluded that:

> For cell culture work – a dealing involving the use of a replication defective, retroviral vector, originally classified as a PC2-NLRD, can be treated as an Exempt dealing after a period of at least 72 hours **AND** the number of total (100%) culture media changes exceeds three (3).

> For animal work – a dealing involving inoculation with a replication defective, retroviral vector, originally classified as a PC2-NLRD, can be treated as a PC1-NLRD (if used in transgenic or knockout, mice, rats, guinea pigs or rabbits) or an Exempt dealing (if used in wild type animals) after a period of 48 hours.

Acknowledgement

Appendix A: Stability of retroviral based vectors systems in tissue culture.

The half-life of virus particles *in vitro* can be influence by many factors including temperature, pH and growth media components. In terms of evaluating the half-life of viruses following transduction protocols the presence of serum in cultures is probably the most important factor. Both mouse and human mouse sera rapidly inactivate the VSV-G-pseudotyped HIV-1 and MLV based viruses. This inactivation can be somewhat attenuated by heat-inactivation of the sera at 56°C (see Table below), consistent with the process being mediated through the classical complement pathway.
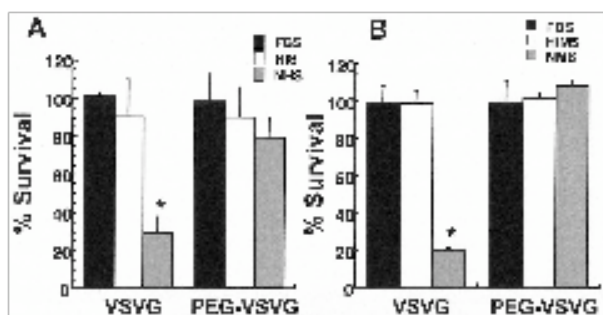
TABLE 2

Half-Lives of Viral Vectors in Human or Mouse Serum

	Half-life (min)	
	HIV-1	MLV
Control		
(without serum)	321.2 ± 62.2	207.0 ± 34.3
Human serum		
(Unheated)	25.3 ± 6.0*	59.2 ± 8.8*†
(Heated)	168.7 ± 17.1†††	147.6 ± 27.4
Normal mouse serum		
(Unheated)	34.9 ± 5.0***	2.24 ± 0.47***·†††
(Heated)	33.5 ± 6.1*·‡	2.04 ± 0.48***·†††
SCID mouse serum		
(Unheated)	94.2 ± 15.6	156.2

(Higashikawa and Chang 2001)

Similar studies have evaluated the effect of mouse and human sera on inactivating virus in comparison to heat inactivated fetal bovine sera (HI-FBS), which suggests that HI-FBS does not inactivate virus.

(Croyle et al. 2004)

Knock-Out Serum Replacement is used for the culture of hESC and iPS cells, there is no information regarding this product in the context of virus inactivation. The differentiation protocols for ESCs variably contain FCS (usually bovine), bovine serum albumin (BSA) or recombinant albumin, with none of these media components likely to cause inactivation of the viral particles.

 The half-life of VSV-G-pseudotyped HIV-1 based recombinant viral particle is variably described as being 321-2 ± 62.2 min (5 and ½ hrs, in the absence of sera, Higashikawa and Chang, 2001) or between 10 to 24 hrs ((Pauwels et al. 2009), but it is not clear what evidence this is based on).

Other factors that will affect the amount of virus remaining in the dish following a transduction protocol will also include the efficiency of the uptake of the virus. Accordingly a dish of cells transduced with 10^6 virus particles would in the best-case scenario (if the transduction has been set up at a multiplicity of infection of 1 (i.e. 10^6 virus particles transfecting 10^6 cells) and 75% of your cells are successively transduced) have approximately 250,000 viral particles remaining. In a 24 hour period these particles would have gone through approximately 4 half-lives with ~15,000 viral particles remaining. A media change comprising 95% of the media would then result in less than 1000 viral particles remaining.

Stability of viral vectors in Animals

As indicated above the reported half-life for HIV-1 based particles varies with indications ranging between 5 to 24 hrs dependent on conditions. VSV-G-pseudotyped HIV-1 vector particles have a half-life of 10.4 ± 1.2 hr at 37°C, 1 to 2 days at room temperature, and ~1 week at 4°C (Higashikawa and Chang, 2001).

However, this is in vitro and therefore unlikely to reflect the situation *in vivo* where the immune system will have a dramatic effect.

Studies on viral clearance have focuses on adenovirus and indicated that the half-life in blood is reduced to less than 2 minutes. The time to clear virus particles from the blood is dependent of various factors with size playing a critical role. Rapid clearance, >99% of an intravenous inoculum, of large viruses occurs within 1 hour (Alemany *et al.* 2000).

Alemany *et al.* demonstrated that an inoculation of 10^{10} transducing units (t.u.) of andenovirus showed a 7 fold reduction in the first 1-5 minutes and reduced to 5×10^2 t.u. within 60 minutes. This was attributed to the efficiency of Kupffer cells in the liver at clearing virus.
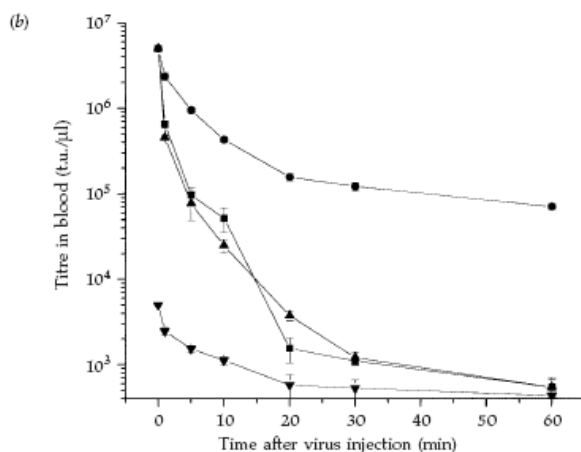

Fig. 2. Adenovirus clearance from blood and the effect of KC depletion and hexon charge. A total of 10^{10} t.u. virus containing a GFP-expression cassette was injected via the vena cava into BALB/c mice in a surgical procedure. At the indicated times post-injection, blood was drawn from the tail into heparinized tubes. Clotting of the tail section was prevented with heparin and bleeding between intervals was suppressed with pressure. The titre at each time-point was assessed by serial dilution of the plasma followed by infection of A459 cells. Transducing units were measured 16 h post-infection. (A) Fluorescence microscopy of wells corresponding to the 1000-fold dilution. The three rows show Adwt, Adwt after GdCl₃ treatment (Adwt + Gd) and AdhN at different times (in min). (B) Quantification of the virus titre (as t.u./µl blood) with Adwt (■), Adwt after GdCl₃ treatment (●), AdhN (▲) and PEGylated Adwt (▼). The t_0 value was calculated as the total injected dose distributed in 2 ml (7·3% body weight) (n = 3 mice).

References

Alemany, R., Suzuki, K. and Curiel, D.T. (2000) Blood clearance rates of adenovirus type 5 in mice. Journal of General Virology **81**: 2605–2609

Croyle, M. A., S. M. Callahan, A. Auricchio, G. Schumer, K. D. Linse, J. M. Wilson, L. J. Brunner and G. P. Kobinger (2004). "PEGylation of a vesicular stomatitis virus G pseudotyped lentivirus vector prevents inactivation in serum." Journal of virology **78**(2): 912-921.

Higashikawa, F. and L. Chang (2001). "Kinetic analyses of stability of simple and complex retroviral vectors." Virology **280**(1): 124-131.

Pauwels, K., R. Gijsbers, J. Toelen, A. Schambach, K. Willard-Gallo, C. Verheust, Z. Debyser and P. Herman (2009). "State-of-the-art lentiviral vectors for research use: risk assessment and biosafety recommendations." Current gene therapy **9**(6): 459-474.