Guidelines on classification of projects involving use of lentiviral vectors in mammalian cell line

The table below gives details of the appropriate classifications to be applied to GM work using lentiviral vectors, following guidance contained in Section 2.11 of the Scientific Advisory Committee for Genetic Modification [SACGM] Compendium of Guidance.

Nature of activity	Class
• **2nd/3rd generation LVV** [must not contain X protein expressing forms of WPRE] • **encoding non harmful genes [eg housekeeping, reporter genes , GFP etc],** • **activity not requiring use of sharps**	**1**
• **2nd/3rd generation LVV [as above]** • **encoding for gene modulators/modifiers or similar genes** • **not involving over expression of any gene that could be considered oncogenic by definition**	**Class 2** but ensure it is covered by C0701 which has been notified
Any use of LVV outside of the above parameters	**Class 2 and to be notified to HSE**

Note A copy C07-01 can be obtained by contacting the local GM committee Chair or the University Biosafety Adviser

Relevant Extracts from SAGCM Guidance

Hazard Assessment Summary
Hazards associated with these vectors are summarised as: stable expression of transgenes, insertional mutagenesis and potential for generation of replication competent virus. Replication defective vectors that cannot infect human cells can generally be considered class 1. For replication defective retroviruses and lentiviruses capable of infecting human cells, if the risk assessment demonstrates they are adequately attenuated, it is possible to designate the activity as class 1 :- Factors supporting this classification will include: low risk of generation of RCV (e.g. a third generation packaging system), self inactivating (SIN) LTR and non-harmful insert. However, contaminated sharps represent a significant hazard (see paragraph 31), and their use should be excluded for vectors that can infect human cells, if the activity is to be designated class 1.

Lentiviral Vectors.
Lentiviral vectors have become widely used due to their ability to infect non-dividing cells, which gives them an advantage over oncogenic retroviral vectors for certain applications. Furthermore, unlike oncogenic retrovirus vectors, transformation has not been seen when using lentivirus systems in a broad range of **in vitro** studies and animal

studies using both **in vivo** and **ex vivo** protocols. However, in common with AAV and MLV vectors, liver tumours have been observed following administration of lentiviral vectors to foetal or neo-natal animals. This is based on limited data and the mechanism by which these tumours arise has not been elucidated.
For example, it is not clear whether or not this is due to vector activity.

"First Generation" lentiviral vectors resemble third generation oncogenic retroviral systems, and are composed of a transfer vector containing all viral components except **gag, pol** and **env** which are provided **in trans** by twohelper constructs. Several of the lentiviral accessory genes are deleted in"Second Generation" transfer vectors **(vif, vpr**, **vpu** and **nef**) since they are not required for **in vitro** replication and the products they encode have cytotoxic activities. In "Third Generation" vectors, the **tat** gene is also deleted and the Tat-responsive promoter present in the 5' LTR is replaced with heterologous promoters, for example with the Rous sarcoma virus U3 region. Additional biosafety is achieved by deletion of the **rev** gene from the transfer vector and expressing this from a third packaging construct as well as employing the SIN principle (see below).

For more detailed information go to Section 2.11 of
http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/part2.pdf