DRAFT

Policy on Research Utilizing Retro/Lenti Virus Transduced Cells

According to current Health Canada guidelines, research with cells transduced with replication defective retroviral or lentiviral vectors must be carried out with Biosafety Level 2 (BSL 2) and BSL 2+3 containment conditions, respectively, when live virus is used. The same is true if live virus is administered directly into animals. Furthermore, any work involving any type of live viral vector containing a known oncogene must be done at BSL 3 whenever the vector is infectious for human cells. According to Health Canada, these strictures are necessary because, even though the viral vector may be replication defective, endogenous retroviruses residing within the transduced cells could package the nascent viral RNA as pseudotyped infectious particles. Both amphotropic and xenotropic retroviruses from different species are capable of infecting human cells.

Based on recent discussions, a partial compromise has been reached with Health Canada concerning appropriate containment levels for retrovirally transduced cell lines. If the transduced gene is known to promote cell survival or alter cell cycling in favour of proliferation (as in the case of an oncogene), then BSL 2 and BSL 2+3 containment must be maintained for live retroviral and lentiviral vector work, respectively, especially if the vectors are capable of infecting human cells. Normally, once stable transductants have been selected, cells containing a gene that does not affect the cell cycle or proliferation status (such as a reporter gene), are maintained at Level 2 or Level 2+3 as are animals injected with these cells. However, the level of containment could be dropped for such cells if transduced, stably selected cells are demonstrated not to be producing virus. This would need to be demonstrated be means of quantitative RT-PCR with appropriate standards to confirm assay sensitivity. Virus should be concentrated from clarified cell free supernatants by ultracentrifugation before RNA extraction. If the virus is undetectable in this assay, a BSL 2 or BSL 2+3 cell line could go to BSL 1 (assuming the cell line itself does not require a higher containment level, as in the case of transformed cells with an activated oncogene). Animals injected with these reclassified cells could also be handled at BSL 1. Positive detection of the virus in culture supernatants would require the original containment level to be maintained.

Note that this “dropdown” option does not apply to immunocompromised mice repopulated with primary human or nonhuman primate, unmodified primary or retro/lenti virus modified cells. For those mice the containment must not be lower than BSL 2 (the standard for handling any primary human or NHP material). If the primary cells are known to be infected with a BSL 3 agent (HIV, SARS, HTLV-I/II or B virus, for example) then the cells must be contained at BSL 3.