OCCUPATIONAL HEALTH CONSIDERATIONS FOR WORK WITH LENTIVIRAL VECTORS

GARY R. FUJIMOTO, M.D.

OCCUPATIONAL MEDICINE CONSULTANT

OCTOBER 6, 2014

Disclosure: Lecture includes off-label use of antiretroviral medications

VIRAL VECTORS

Definition:

Viruses engineered to deliver foreign genetic material (transgene) to cells

Many viral vectors deliver the genetic material into the host cells but not into the host genome where the virus replicates (unless replication incompetent)

Retroviral and lentiviral vectors deliver genetic transgenes into the host chromosomes

LENTIVIRAL VECTORS

- Human immunodeficiency virus (HIV) is a lentivirus that infects both dividing and non-dividing cells
- Use of the HIV virus as a viral vector has required the reengineering of the virus to achieve safe gene transfer
- Since HIV normally targets CD4 cells, replacing the HIV envelope gene with vesicular stomatitis virus glycoprotein (VSV-G) expands the infectious range of the vector and modes of transmission

LENTIVIRAL VECTORS

- Remember: replication deficient lentiviral vectors integrate the vector into the host chromosomes
- 3rd and 4th generation constructs unlikely to become replication competent with enhanced safety due to self-inactivating vectors (however, consider present or future HIV infection)
- Replication deficient lentiviral vectors should be regarded as single-event infectious agents
- Many researchers regard these agents as relatively benign although transgene integration does occur with generally unknown effects

LENTIVIRAL OCCUPATIONAL EXPOSURES

- Lentiviral (LV) risks in research settings primarily involve the inadvertent transduction of the lab worker
- These include the potential harmful effects of the transgene, insertional mutagenesis, or the activation of neighboring genes from vector integration or generation of replication competent lentivirus (RCL) following an existing or subsequent HIV infection

GENE THERAPY

- 5 children (out of 20) treated with retroviral vector containing IL2RG gene for X-linked SCID developed leukemia 3-6 years after treatment (insertional mutagenesis)
- Vector inserted into the chromosome near the LMO2 gene which has been implicated in several Acute Lymphoblastic Leukemia (ALL) translocations
- 4/10 gene therapy patients with Wiskott Aldrich syndrome (X-linked heme disorder) developed ALL
- None of the 20 adenosine deaminase (ADA) SCID patients developed ALL

RNA INTERFERENCE (RNAi)

- RNAi was chanced upon when genetic engineers sought to insert the purple gene into a purple petunia to create a deeper purple flower
- This resulted in a white pigment-free flower which confounded the researchers
- This was subsequently discovered to be due to double stranded RNA (dsRNA) which is not normally found in human cells

SHORT INTERFERING RNA (siRNA)

- Cytoplasmic delivery of short interfering dsRNA (siRNA) is normally due to viral and other exogenous sources
- Human cells identify this as foreign and cleave it into siRNA or short 21-23 nucleotide long sequences then the RNA induced silencing complex (RISC) then unwinds and separates the dsRNA
- The antisense single strand (or guide strand) targets complementary mRNA sequences where it binds and inactivates them shutting down protein synthesis
- This is why the purple gene was inactivated

siRNA

- Lentiviruses are now being used since siRNA don't cross cell membranes
- May provide new ways to silence cancer cells, viruses (Ebola, HBV, HPV, SARS), metabolic disorders, neurodegenerative diseases, and inherited genetic diseases
- Also allows for rapid drug target discovery and in vitro validation of these targets in cell culture
- Problems include 10% off-target effects

POTENTIALLY HAZARDOUS TRANSGENES

- Oncogenes or tumor suppressors
- Growth regulators
- Targets having important cellular functions
- Targets focused on the host-immune system
- Small interfering (si) or short-hairpin (sh) RNA

  that affect the above functions
- Transgenes without known targets carry unknown risks

LENTIVIRAL OCCUPATIONAL EXPOSURES

- LV and retroviral vector exposures, particularly if associated with a hazardous transgene (e.g., an oncogene or toxin), should consider use of an antiretroviral agent
- Treat rapidly since reverse transcription and integration occurs in 12-24 hours (or shorter)
- These can include the reverse transcriptase and integrase inhibitors, but not the protease inhibitors or CCR5 receptor antagonists
- Non-lentiviral retroviral vectors cannot be treated with non-nucleoside reverse transcriptase inhibitors

RECOMMENDATIONS FOR HANDLING LENTIVIRAL AND RETROVIRAL VECTORS

- Use advanced lentiviral vector systems
- Avoid mixing systems
- Review potential for replication competent virus
- Avoid sharps and glass – anesthetizing animals
- Use PPE to avoid exposures to eyes, nose and mouth
- Containment within BSC's when possible aerosol generation

RECOMMENDATIONS FOR HANDLING LENTIVIRAL AND RETROVIRAL VECTORS

- Consider risk for mutagenesis or toxic properties of transgene
- Consider risk from animals treated with LV
- Consider risk of viral shedding in immunodeficient animals
- Consider present or future risk for HIV in lab personnel along with confidential testing (pre-placement and serial testing)
- Maintain record of vectors especially post-accident
- Consider antiretroviral treatment post exposure

ANIMAL BIOSAFETY ISSUES WITH LENTIVIRAL VECTORS

- Recent studies with 3rd generation self-inactivating LV showed infectious LV recoverable on dry plastic for 24 hours and in vector-spiked soiled bedding for up to 72 hours
- Infectious virus also found at the injection site (tail) for up to 24 hours (attributed to vector leakage upon needle removal)
- Protocols vary on when to go to ABSL-1 (usually 1-7 days for non-hazardous transgenes), but usually include disinfecting inoculation site and holding container/cage with 70% ethanol

RECOMMENDATIONS FOR HANDLING LENTIVIRAL AND RETROVIRAL VECTORS

- For many experiments BL-2 or enhanced BL-2 are appropriate (consider mucous membrane and aerosol hazards for VSV-G pseudotyped virus including retroviruses)
- Some experiments may warrant BL-3 practices
- Recommend disposable lab coat, gloves, safety glasses and containment with biosafety cabinets
- Transport to avoid generation of splatter/aerosol

VECTOR/TRANSGENE HAZARDS

- Problems include what to monitor and for what length of time due to the potential for long latent periods
- Need to consider the consequences of exposure to the genetic insert when performing biosafety reviews and the additional issues with off-target effects or generation of replication competent virus and viral titer
- Need to proactively train all staff to understand potential risks with these agents and on ways to prevent exposures
- Need to develop PEP protocols **PRIOR** to an exposure
- Need to develop system to report and monitor exposures

SUMMARY

- Lentiviral vectors are single event replications that insert the transgene into the host's chromosomes
- VSV-G pseudotyping broadens the range of infected cells and increases the modes of transmission
- Planning for post-exposure prophylaxis needs to be planned in advance and initiated quickly
- Most physicians are not familiar with lentiviral vectors and need to be educated in advance regarding treatment options