OCCUPATIONAL HEALTH CONSIDERATIONS FOR WORK WITH VIRAL VECTORS

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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 cytoplasm where the virus replicates (unless replication incompetent)
NON-RETROVIRAL VECTORS

- Adenovirus widely used since replication incompetent vectors can generate high titers infecting both dividing and non-dividing cells and can be administered by aerosol

- However since integration into the host genome does not occur, gene expression is transient

- Adenoviral vectors can generate an immune response to viral proteins
VECTOR HAZARDS

- Ornithine transcarbamylase deficiency is a genetic disorder that leads to potentially fatal accumulation of ammonia in infants.

- Jesse Gelsinger, an 18 y.o. with a mild form of this disorder, was entered into a clinical trial where he received an adenoviral vector with an OTC transgene.

- He died 4 days later after a severe inflammatory response lead to disseminated intravascular coagulation and multiorgan failure possibly due to a previous exposure to wild type virus.

- Prior studies in primates suggested such treatment may elicit a cytokine cascade.
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
- Replication deficient lentiviral vectors should be regarded as single-event infectious agents
- Many researchers regard these agents as relatively benign
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).
Gene therapy is a technique for correcting defective genes responsible for disease.

While genes could be repaired, swapped or up/down regulated, most current methods involve inserting normal genes into non-specific regions of the genome.

Targets genetic deficiencies (e.g., severe combined immunodeficiency syndrome - SCID) or cancer cells (e.g., advanced metastatic melanoma).
Gene Therapy

- 5 children 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
- 1/10 gene therapy patients with Wiskott Aldrich syndrome (X-linked heme disorder) developed ALL
- None of the 20 adenosine deaminase (ADA) SCID patients developed ALL
- Lentiviral vectors may have reduced risks
RNA INTERFERENCE (RNAi)

- Human genome project led to sequencing of the entire human genome and to multiple other organisms
- Knowledge about gene function through generation of transgenic animals is costly and time consuming
- The alternative with selective gene silencing has been facilitated through the discoveries of RNA interference by Fire and Mello (Nobel prize 2006)
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 by Dicer, a ribonuclease III enzyme.

- These short duplexes are incorporated into a protein complex called the RNA-induced silencing complex (RISC).
siRNA

- RNA induced silencing complex (RISC) then unwinds and separates the dsRNA through the protein Argonait 2 contained within the RISC complex
- The antisense single strand (or guide strand) targets complementary mRNA sequences where it binds and inactivates them shutting down protein synthesis
- When siRNA is delivered to the cytoplasm, the effect is relatively transient lasting up to 7 days in rapidly dividing cells and up to several weeks in resting cells
- This is why the purple gene was inactivated
SHORT HAIRPIN RNA (shRNA)

- Another pathway involves a dsRNA which is delivered to the nucleus via LV and integrated into the host genome which generates a short hairpin shaped dsRNA
- These are exported to the cytoplasm where they enter the same pathway as siRNA
- These sequences require less specific base pair binding than siRNA and can lead to increased off-target effects
- Nuclear integration leads to long-term gene knock down effects
shRNA

- Lentiviruses are now being used since shRNA are highly charged and don’t cross cell membranes
- May provide new ways to silence cancer cells, viruses (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
ONCOGENES

- Tool in cancer research to modify cells to express oncogenes with transfer of modified cells back to animal
- Risk of accidental exposure with an oncogenic vector to laboratory staff
- Tumors that are the result of hazardous vector/transgenes will be marked by the integrated vector
- Structural information about the vectors (the DNA sequencing) needs to be maintained by the lab to determine whether a retroviral transduction is responsible for a future tumor
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
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
- 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
POST EXPOSURE PROPHYLAXIS FOR LENTIVIRAL EXPOSURES

- HIV transduction (reverse transcriptase (RT) and integration into the host genome) takes 12-24 hours (possibly shorter)

- Nucleoside and non-nucleoside reverse transcriptase along with integrase inhibitors blocked viral replication in lentiviral exposures even 12 hours post-exposure

- Murine leukemia viral (retroviral) vectors were inhibited by nucleoside but not non-nucleoside RT inhibitors

- Early treatment might reduce transduction of potentially hazardous vectors, but needs to be planned in advance and duration of treatment not established

- Speed in initiating PEP treatment is critical
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 particularly if engrafted with permissive cells

• Consider risk of viral shedding in immunodeficient animals

• Consider present or future risk for HIV in lab personnel along with confidential testing

• Maintain record of vectors especially post-accident
ANIMAL BIOSAFETY ISSUES WITH LENTIVIRAL VECTORS

In vivo studies require housing at BL-2 for the life of the study UNLESS

- The transgene is non-oncogenic
- The animal/host is non-permissive for HIV-1
- The vector system is third generation or higher with low risk for creating replication competent lentivirus
- Containment may be lowered from BL-2 to BL-1 anywhere from 1-7 days as determined by the IBC
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