

# OCCUPATIONAL HEALTH CONSIDERATIONS FOR WORK WITH VIRAL VECTORS, RNA INTERFERENCE AND GENE EDITING

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# 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
- No treatment options for exposures

# 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

- 3<sup>rd</sup> and 4<sup>th</sup> generation constructs unlikely to become replication competent by splitting vector and packaging system into 4 plasmids (however, consider present or future HIV infection) and by self-inactivating vector systems
- Remember: replication deficient lentiviral vectors integrate the vector into the host chromosomes
- Replication deficient lentiviral vectors should be regarded as single-event infectious agents

# LENTIVIRAL VECTORS

- Commercial kits allow for vector insertion by those not fully knowledgeable about these vector systems
- 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

- 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)

# GAMMA RETROVIRAL GENE THERAPY

- 5/20 children treated with retroviral vector containing IL2RG gene for X-linked SCID developed leukemia 2 to 5.5 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
- 7/10 gene therapy patients with Wiskott Aldrich syndrome (WAS) X-linked heme disorder developed ALL or AML
- None of the 34 adenosine deaminase (ADA) SCID patients developed ALL

# ONGOING GENE THERAPY TRIALS WITH SELF-INACTIVATING (SIN) VECTORS

- X-linked SCID (SIN  $\gamma$ -RV) 8/9 with immune recovery
- X-linked SCID (SIN-LV) 5 >2 y.o. and 3 infants all with immune recovery
- ADA-SCID (SIN-LV) 5/5 with clinical improvement
- WAS (SIN-LV) 12/13 with clinical improvement

Note – no adverse events in above trials to date with lower insertion near proto-oncogene sites with SIN-LV, but too early to know if completely safe

# CANCER THERAPY

- Traditional chemotherapy for relapsing ALL has low success rates (<25% remission) with median response: 1-2 months
- Study involving relapsed ALL patients treated with a CD-19 directed chimeric antigen receptor T cell lentiviral vector showed complete remissions in 90% (27/30 children and adults) with 19 remaining in remission (probability being relapse-free at 6 months 73%) with 1 AML
- All developed the cytokine-release syndrome with elevated IL-6 levels and 8 with severe symptoms
- Follow-up period has been for 2-24 months

# 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 19-25 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 Argonaut 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

# CRISPR-CAS9 GENE EDITING

- CRISPR (Clusters of Regularly Interspaced Short Palindromic Repeats) is a new gene editing system that relies on an enzyme called Cas-9 to target sites on DNA where it cuts and replaces genes or desired genetic sequences
- System has the potential to alter defective genes, create modified plants and animals or eliminate certain pathogens
- CRISPR is inexpensive, quick and relatively easy to use

# CRISPR CONCERNS

- Concerns include making genetic modifications to humans, generating altered species, inserting into off-targeted sites and making species wide changes through gene drive (where both chromatids of the chromosome are altered therefore transferring the trait to all subsequent generations)
- CRISPR can be delivered to cells by a variety of viral vectors including lentiviral vectors
- If lentiviral vectors are used with CRISPR systems, early use of antiretroviral PEP can block this insertion

# POTENTIALLY HAZARDOUS TRANSGENES

- Oncogenes or tumor suppressors
- 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
- CRISPR or other gene editing systems
- Transgenes without known targets carry unknown risks

# RISK ASSESSMENT FOR LENTIVIRAL VECTOR EXPOSURES

- Assess vector system and potential for generation of replication competent lentivirus
- Evaluate the nature of the transgene
- Consider vector titer and total amount of vector
- Consider the biological containment of the animal host (if performing animal studies)
- Consider the potential routes of exposure

# 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
- Initiate rapidly since reverse transcription and integration occur within 12-24 hours or less
- Recognize that LV gene transfer for X-linked SCID have lead to false positive HIV tests by PCR

# EAGLESON RECOMMENDATIONS

- Eagleson Committee on lentiviral vector exposures (meeting in June 2015) recommends offering immediate treatment for all lentiviral vector exposures involving percutaneous or mucous membrane routes (regardless of the vector) due to concerns for potential long-term tumor induction (publication pending in JOEM)

- Recommended use of one or two drugs:

Tenofovir( NtRT) 300 mg once daily for 7 days and/or

Raltegravir (Integrase inhibitor) 400 mg twice daily for 7 days



# RECOMMENDATIONS FOR HANDLING LENTIVIRAL AND RETROVIRAL VECTORS

- Use advanced lentiviral vector systems
- Avoid mixing commercial 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

- Studies with 3<sup>rd</sup> 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, but usually include disinfecting the cage and applying 70% ethanol to the inoculation site with

# 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

- 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