

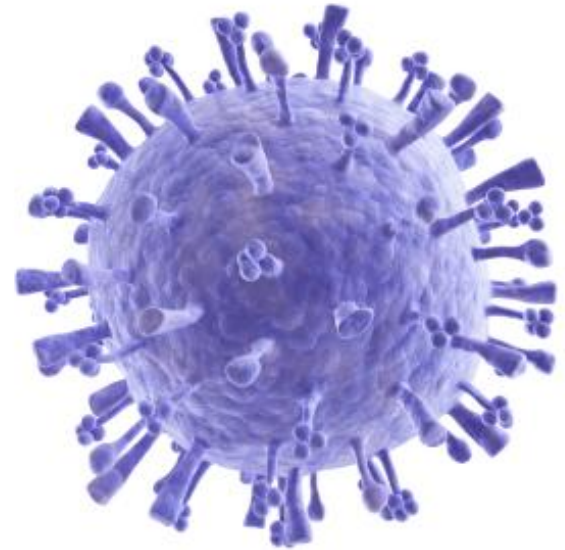
## Biosafety Concerns of Viral Vectors—Focus Lentivirus

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May 5, 2011

# Biosafety Risks of Lentivirus and Other Viral Vectors

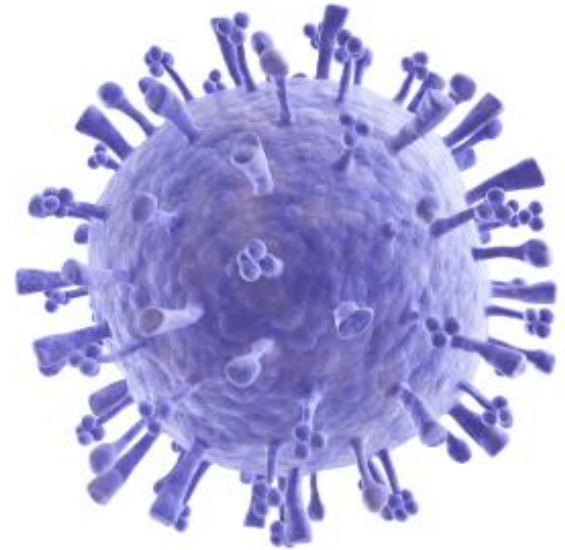
- Recombinant DNA Advisory Committee (RAC)
- 2006 reviewed biosafety of Lentivirus System



# Biosafety Risks of Lentivirus and Other Viral Vectors

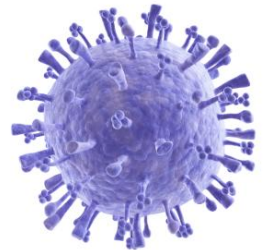
- ▣ Potential for generation of replication competent virus
- ▣ Potential for oncogenesis

Risks are mitigated by the nature of the vector system and its safety features or exacerbated by the nature of the transgene insert encoded by the vector.



# Criteria For Risk Assessment of Lentivirus Vectors

- ❑ The nature of the vector system and the potential for regeneration of replication competent virus from vector components.
- ❑ The nature of the transgene insert (e.g. known oncogenes or genes with high oncogenic potential merit special care)
- ❑ The vector titer and the total amount of vector.
- ❑ The inherent biological containment of the animal host, if relevant
- ❑ Negative RCL testing



# Nature of the Vector System

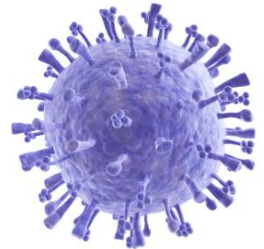
## ▣ General containment considerations

### ■ HIV-1, HIV-2, and SIV

- Wild-type: BSL2+ to BSL3
- Vector Systems: BSL2 (rarely BSL2+)

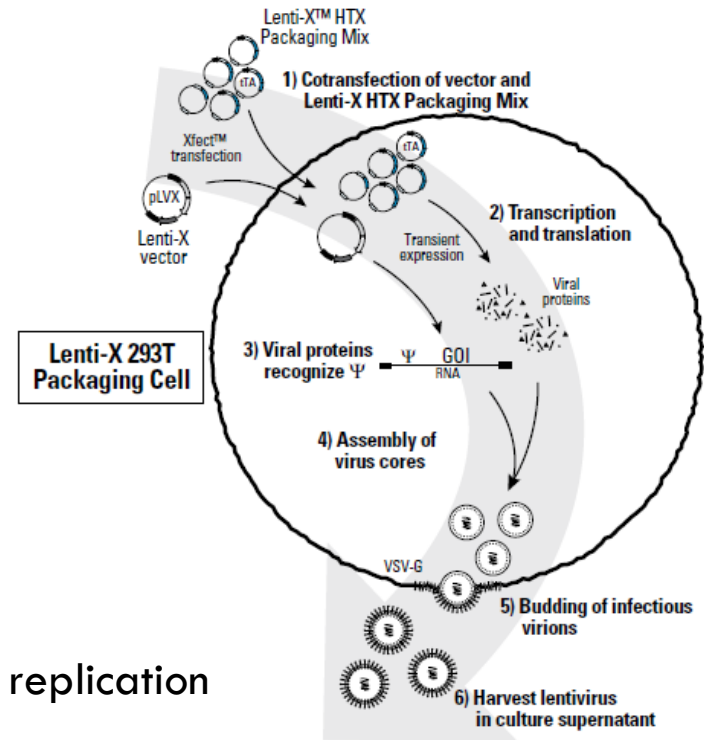
### ■ FIV, BIV, and EIAV

- Wild-type: BSL1
- Vector Systems using VSV-G or other coat protein which broadens tropism to include mammalian: BSL2



# Nature of the Vector System

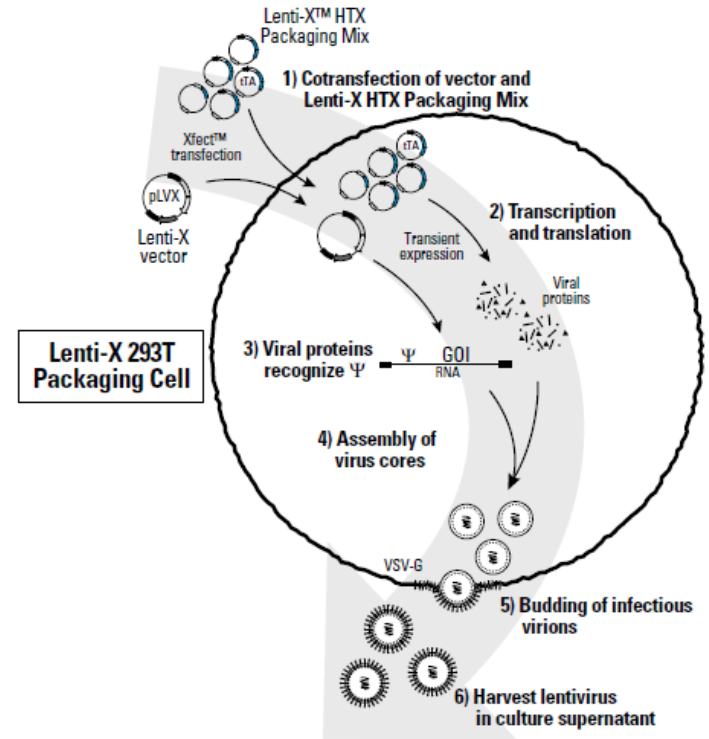
- 2<sup>nd</sup>, 3<sup>rd</sup>, or 4<sup>th</sup> generation viral systems
  - Self-inactivation (SIN) decreases possibility of RCL and insertional mutagenesis
  - Deletion of viral proteins
    - 2<sup>nd</sup> generation (5 of 9 HIV-1 genes eliminated)
    - 3<sup>rd</sup> generation (only gag, pol, and rev remain and a chimeric 5'LTR)
    - 4<sup>th</sup> generation additional changes improving safety and viral production (many proprietary)
  - Separate packaging onto multiple plasmids (3 to 5)
  - Elimination of TAT expression that is required for viral replication



# Nature of the Vector System

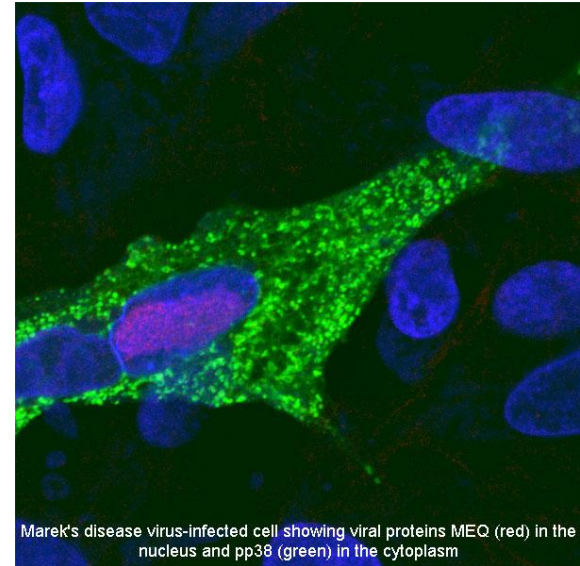
## Concerns

- Mixing and matching systems, generations, and packaging cell lines
- Experience of Lab personnel working with virus and at BSL-2
- Special cases using 2<sup>nd</sup> generation viral system



# Nature of the Insert

- Concerns
  - Accidental exposure—Infectious and Integratable
    - Accidental Injection
    - Splash to mucous membranes, cuts
- Non-Oncogene, non-viral inserts
  - Insertional Mutagenesis
- Oncogene or Potential Oncogene and viral inserts
  - Insertional Mutagenesis
  - Integration and Expression of Oncogene



# Titer, Amount, and Animal Use

## Virus Titer and Total Amount of Virus

- Concerns
  - High titer
    - exposure more likely to result in infection and expression of insert
  - Large volume (greater than 10L)
    - exposure due to handling of large volumes during production
    - RCL event

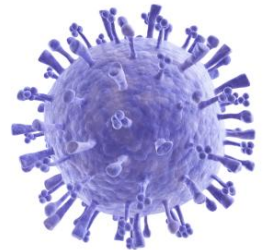
## Biological Containment of Animal Host

- Concerns (post-injection, injections at BSL2)
  - Non-permissive host—ABSL1
  - Special cases will increase standard ABSL containment—ABSL2
    - Host permissive for lentiviral replication
    - Host engrafted with human cells

# Replication Competent Lentivirus Testing

## □ RCL testing

- Required by FDA for use in human clinical trials
- Not required for non-clinical, small volume, and low-risk genes
- Based on risk assessment, the IBC may require RCL testing if larger volumes, high-risk genes, or vector system increase risk of RCL
  - 2006—Had to consider laboratory expertise for working with infectious lentivirus as positive control may increase risk to the investigator compared to the test material
  - P24 Elisa



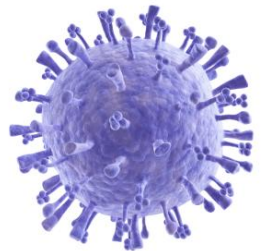
# Spills

## □ Disinfectants—

- Choose disinfectant based on agent sensitivity and contact time
- 10% Bleach or phenolic disinfectants for a VSV-G pseudotype

## □ Inside Biosafety Cabinet (BSC)

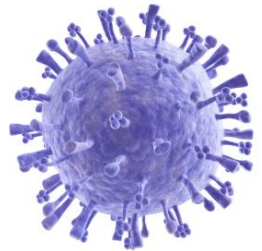
- Spray with disinfectant and wipe up spill with paper towels
- Dispose of paper towels to biohazard container (inside BSC)
- Change to new gloves before continuing work
- Disinfect thoroughly upon completion of work



# Spills

## ▣ Outside BSC

- Cover with paper towels, pour disinfectant over spill, allow a 10' contact time, clean, and repeat (Dispose of materials in Biohazardous Waste Container)
- Inform others in vicinity that a spill has occurred ASAP, do not leave spill unattended
- Wash hands and replace any PPE that has been exposed
- Report to Supervisor, consult BSO if exposure possible
- Evaluate exposure risk



# Exposures

## ❑ IBC Research Accidents and Spills

policy <http://www.ohsu.edu/xd/about/services/integrity/policies/policy-detail.cfm?policyid=1511935>

## ❑ Splash to mucous membranes

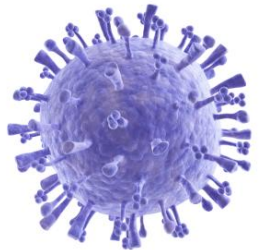
- Rinse eyes, mouth and nose depending on exposure site with water for 10 minutes

## ❑ Accidental Injection

- Encourage bleeding and wash site with soap and water

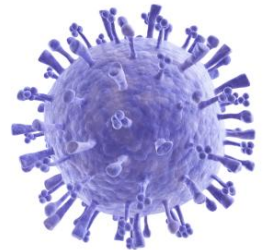
## ❑ After Initial Response

- Report to Supervisor and BSO
- Go to Employee Health or ED for further evaluation
- Complete accident report: <https://ozone.ohsu.edu/wsirs/>



# NIH rDNA Guidelines, BMBL, and Your Lab

- Guidelines used by IBC and the BSO to evaluate protocols and lab practices
  - NIH guidelines
    - [http://oba.od.nih.gov/oba/rac/Guidelines/NIH\\_Guidelines.pdf](http://oba.od.nih.gov/oba/rac/Guidelines/NIH_Guidelines.pdf)
  - BMBL 5<sup>th</sup> edition
    - <http://www.cdc.gov/biosafety/publications/bmb15/>
- Template for Laboratory Specific Lentiviral Manual
  - <http://www.ohsu.edu/xd/about/services/integrity/policies/policy-detail.cfm?policyid=1534831>



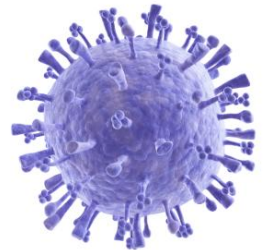
# Risk Assessment of Procedures

## ▣ Work Practices that may require a Risk Assessment (not all inclusive)

- Stereotactic injections

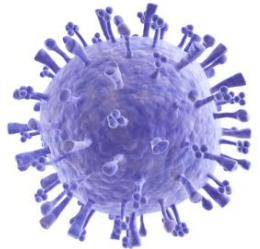
<http://www.ohsu.edu/xd/about/services/integrity/policies/policy-detail.cfm?policyid=1908735>

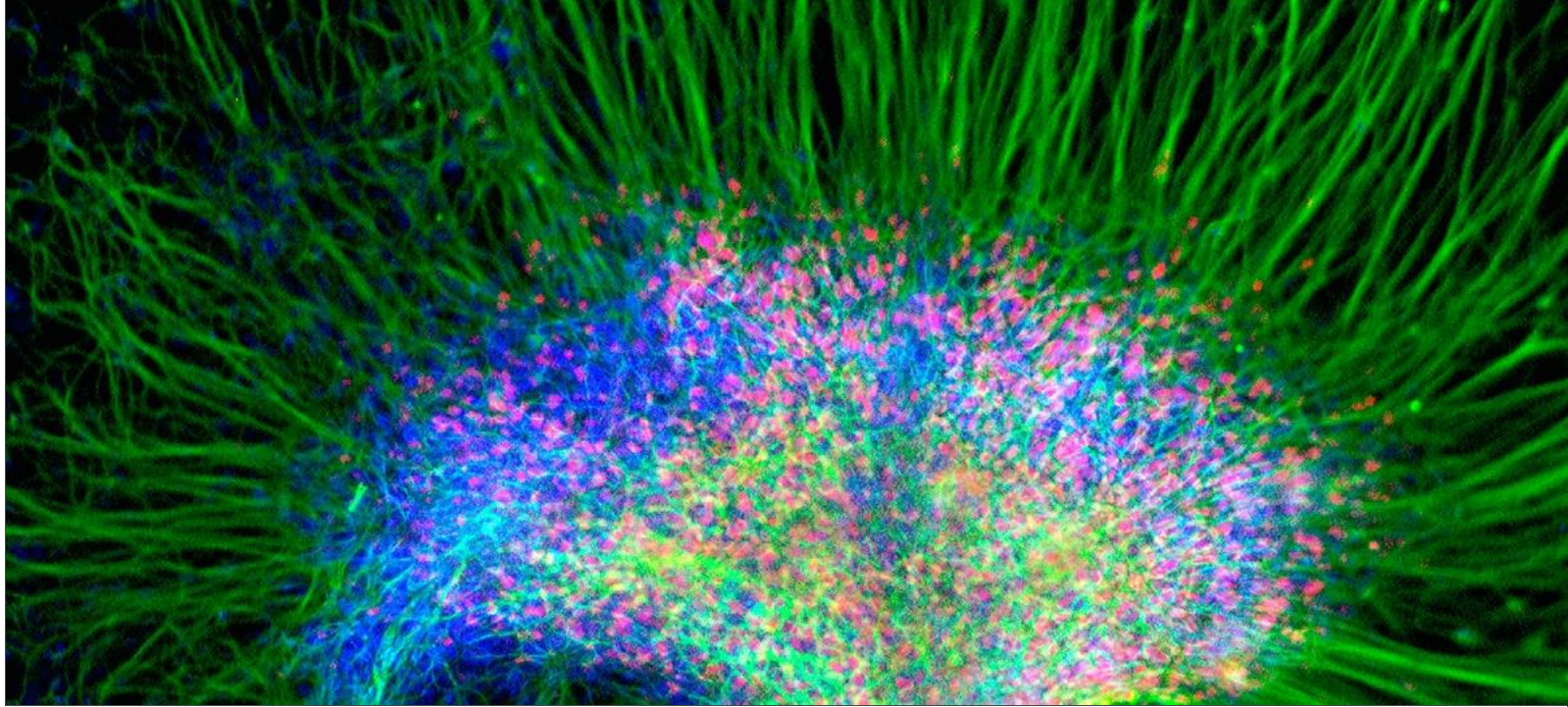
- Animal injections occurring outside BSC
- Work with live virus outside BSC
- Use of sharps



# Training

- ❑ BigBrain Courses ( <https://bigbrain.ohsu.edu/> )
  - General and Laboratory Safety Course
  - Bloodborne Pathogen Training for OHSU Researchers
- ❑ Group training for BSL2 work practices by request
- ❑ DCM/DAR training for ABSL2 and animal injections





Good Luck and Be Safe With Your Research!