Viral Vector Biosafety in Lab and Animal Research

A review on best practices for most commonly used Viral Vectors

Alka Arora, Ph. D
Biorisk Program Manager,
The Hospital for Sick Children

SickKids

Gene Therapy

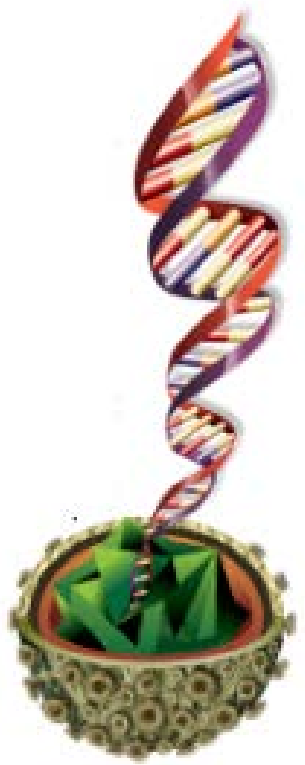
*GT is the introduction of **genes** into existing cells to prevent or cure a wide range of diseases.*

There are several approaches to gene therapy:

- Replacing mutated gene with a healthy copy of the gene e.g. ADA-SCID.
- Inactivating or "knocking out" a mutated gene.
- Introducing a new gene into the cells to help fight a disease, e.g tumor suppressor gene p53.

SickKids

Gene Therapy



Gene therapy utilizes the delivery of DNA into cells, which can be accomplished by

- Naked DNA or DNA complexes- non viral methods
- Recombinant viruses- viral vector

Why Use Viruses?

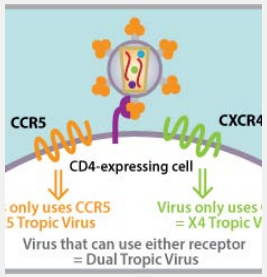
- Viruses are very efficient in transferring their genetic material into host cells
- Specific target cell: depending on the attachment proteins

SickKids

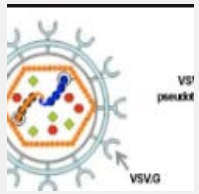
Risk Assessment

Risk Assessment

Risk group of the parent virus from which the vector originated



Tropism: the specificity of a virus for a host tissue



Pseudotyping replace the envelope proteins on the virus by envelope proteins from other viruses to limit or expand the viral tropism

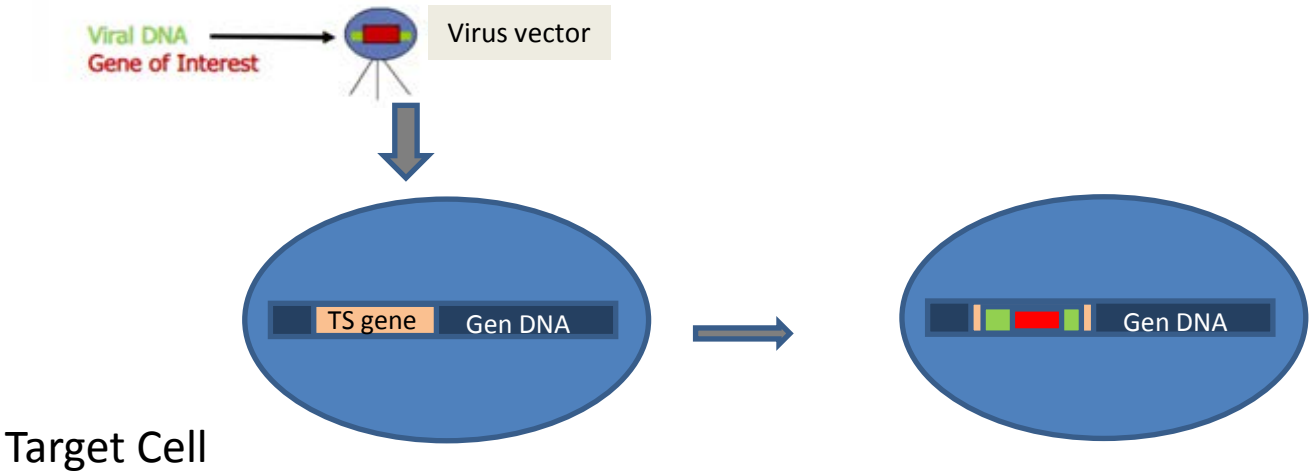
SickKids

Risk Assessment

Risk of Insertional Mutagenesis

Random integration of viral genome may disrupt the endogenous host genes

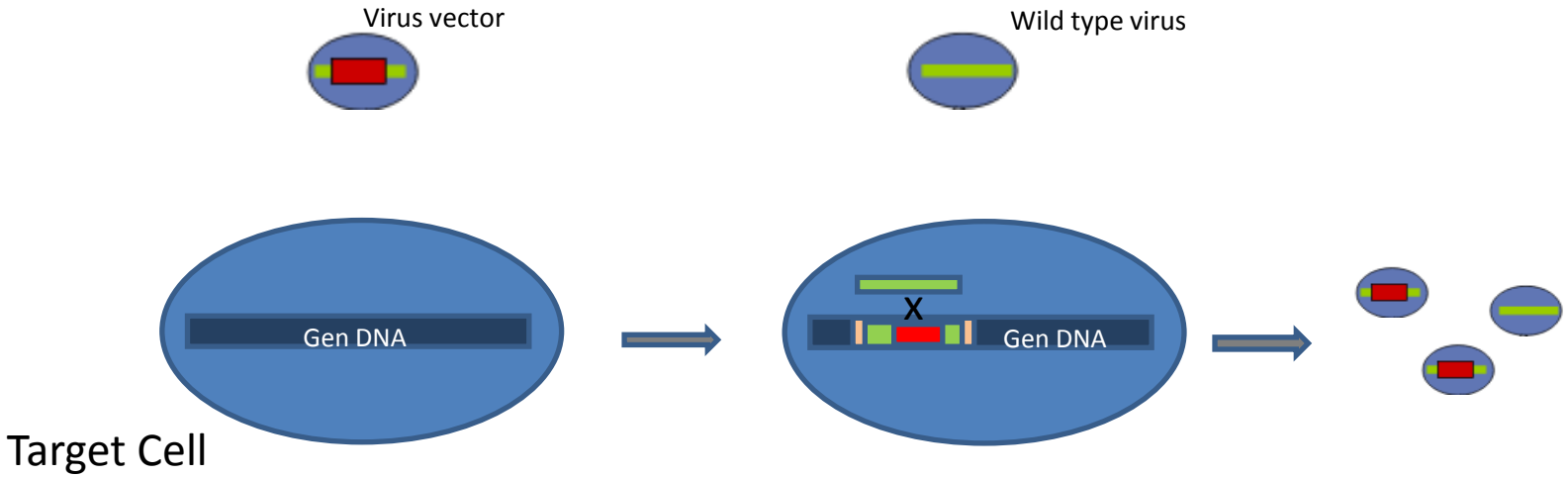
Inactivation of a tumor suppressor gene



SickKids

Risk Assessment

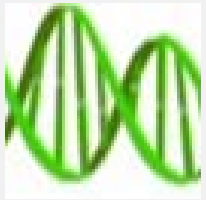
Rescue of Replication Deficient Virus



Vector-transduced cells may be infected by a wild type lentivirus which can potentially act as a helper virus to rescue the integrated vector

SickKids

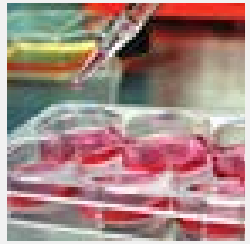
Risk Assessment



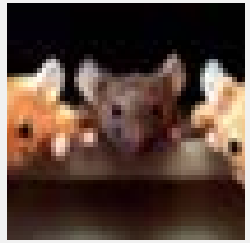
Nature of transgene: Any gene which can significantly alter the cell cycle when over-expressed is a gene of concern.

Stability in the Environment: Lipid enveloped viruses are more sensitive to inactivation and disinfection.

Risk Assessment



Volume generated and Aerosol Generating Procedures: e.g. ultracentrifugation, , homogenization etc.



Animal Host and Manipulation:

-Host, animals engrafted with human cells.

-Use of sharps for administration of viral vectors and surgery etc.

Features	AAV	Adenovirus	HSV	Retrovirus (gamma retroviruses)	Lentivirus	Rabies –G deleted virus
Virus coat	Non-Enveloped	Non-Enveloped	Enveloped	Enveloped	Enveloped	Enveloped
Genome	ssDNA	dsDNA	dsDNA	ssRNA (+)	ssRNA (+)	ssRNA (-)
Risk group	*1*	2	2	2	3	3
Infection range	Dividing and non dividing	Dividing and non dividing	Dividing and non dividing	Dividing	Dividing and non dividing	non dividing cells- Neurons
Host Genome interaction	Mostly non-integrating	Non integrating	Non integrating	Integrate into host genome	Integrates into host genome	Non integrating
Stability in the environment	High stability Remain infectious for a month at RT	High stability 3-8 wks at RT	**Unstable, rapidly inactivated outside the host, highly susceptible to dehydration**	Unstable, rapidly inactivated, sensitive to dehydration	Unstable. Rapidly inactivated outside their hosts	Unstable. Rapidly inactivated outside their hosts
Disinfectants	10% bleach for liquid waste AHP surface disinfection					

SickKids

Viral Vector	Route of Transmission	Host Range	Lab Containment level	Animal work Containment Level
AAV	Ingestion, Mucous membrane Parenteral	Broad host range, infective for many cell types including neurons	CL1- based on risk assessment Packaging: helper plasmid CL 2 : based on risk assessment Packaging: helper virus	CL-1 housing; CL-2 housing in the presence of helper virus.
Adenovirus	Inhalation mucous membrane, parenteral, direct contact,	Broad host range, infective for many cell types	**CL2**	CL2 housing
HSV	Direct contact, Respiratory droplets, mucous membrane exposure Parenteral	Broad host range	**CL2**	CL-2 housing. Amplicon-only is CL-1

Containment Level

Viral Vector	Route of Transmission	Host Range	Lab Containment level	Animal CL
Retrovirus	Parenteral inoculation, Mucous membrane exposure contact exposure of broken skin	Ecotropic Pantropic- VSV-G pseudotyped	CL-1 (ecotropic), CL-2 (amphotropic)	CL-1 housing for ecotropic, CL-2 for amphotropic
Lentivirus	Mucous membrane, Parenteral, Direct contact,	Broad host range, infective for many cell types	CL2 : based on risk assessment CL2+: based on risk assessment	In rodents without human cells present: CL2
Rabies G deleted virus	Parenteral injection Mucous membranes Contact exposure of broken skin	Broad host range	CL2	CL2