



GENES OUT, VIRUS IN

making designer virus to breach species barrier



Dianne Langford, PhD
Lewis Katz School of Medicine
at Temple University
Department of Neural Sciences
Philadelphia, PA

Studying Human Disease in Rodent Models

Cannot use humans

Breed identical mouse strains

One mouse year equals about 30 human years, breed rapidly

Can be genetically manipulated to mimic some aspects of human disease



Ecotropic Viruses

viruses that will only infect cells in its natural host

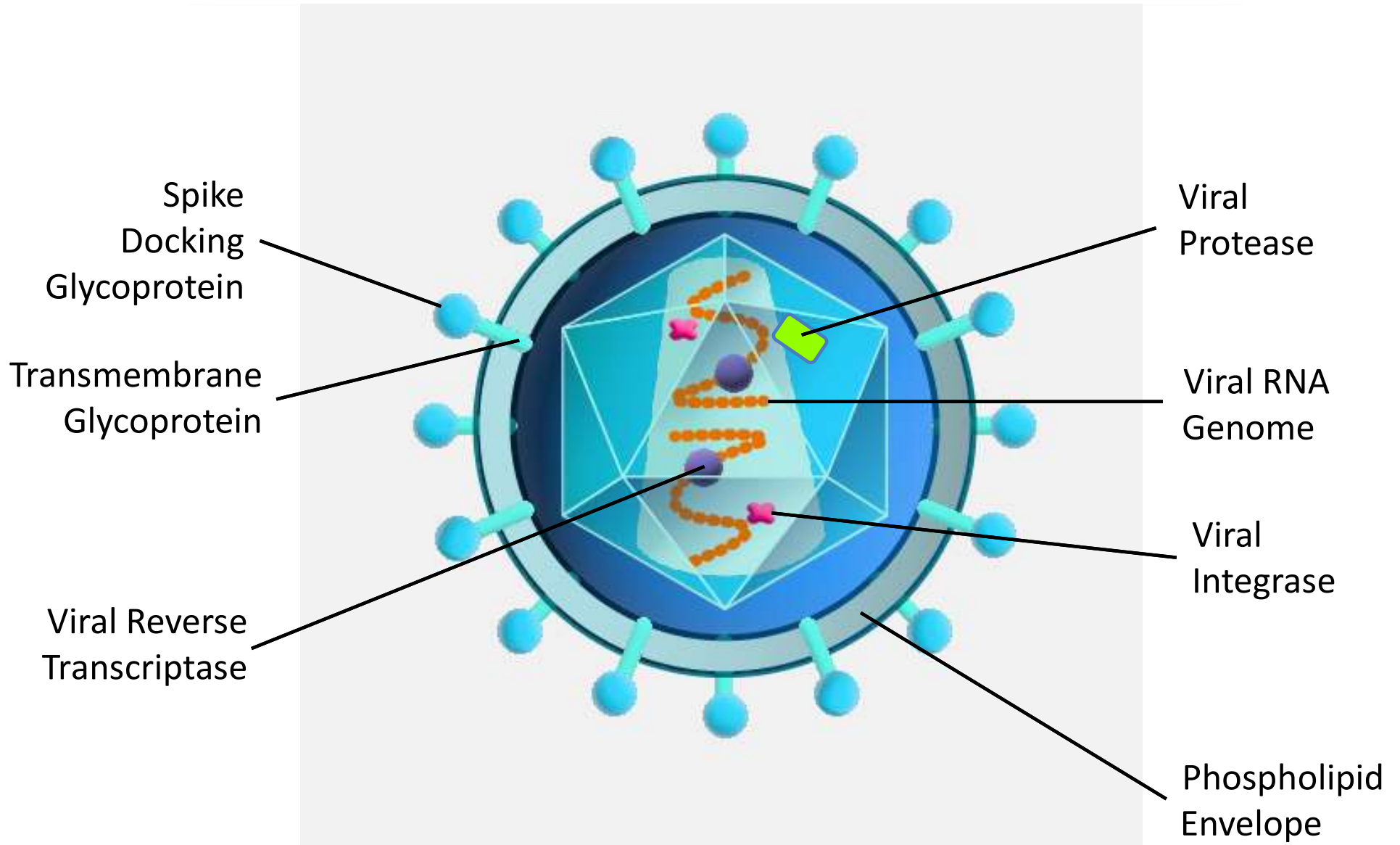
Amphotropic Viruses

viruses that will infect mammalian cells

Pantropic Viruses

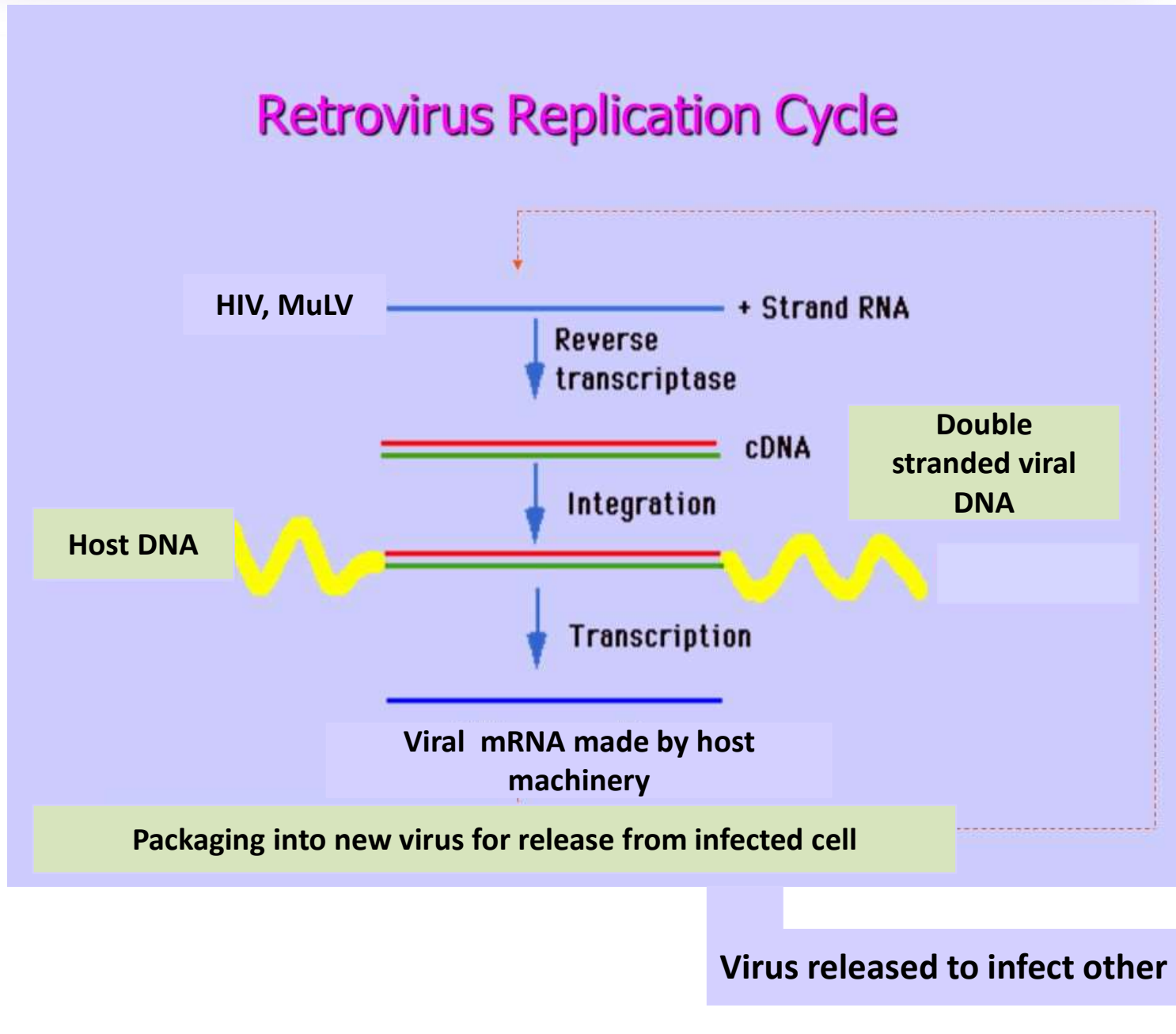
viruses that will infect almost all cells (tissues)

Retrovirus Structure

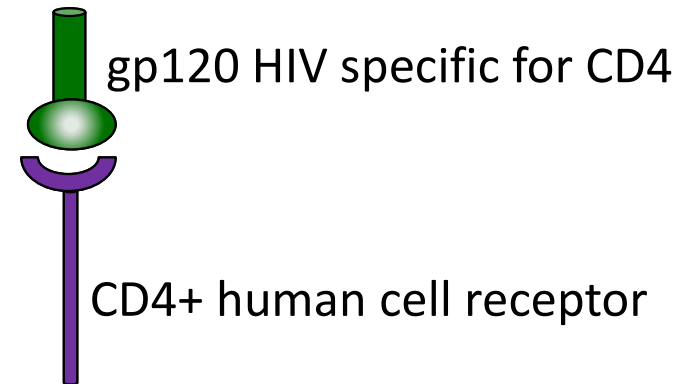
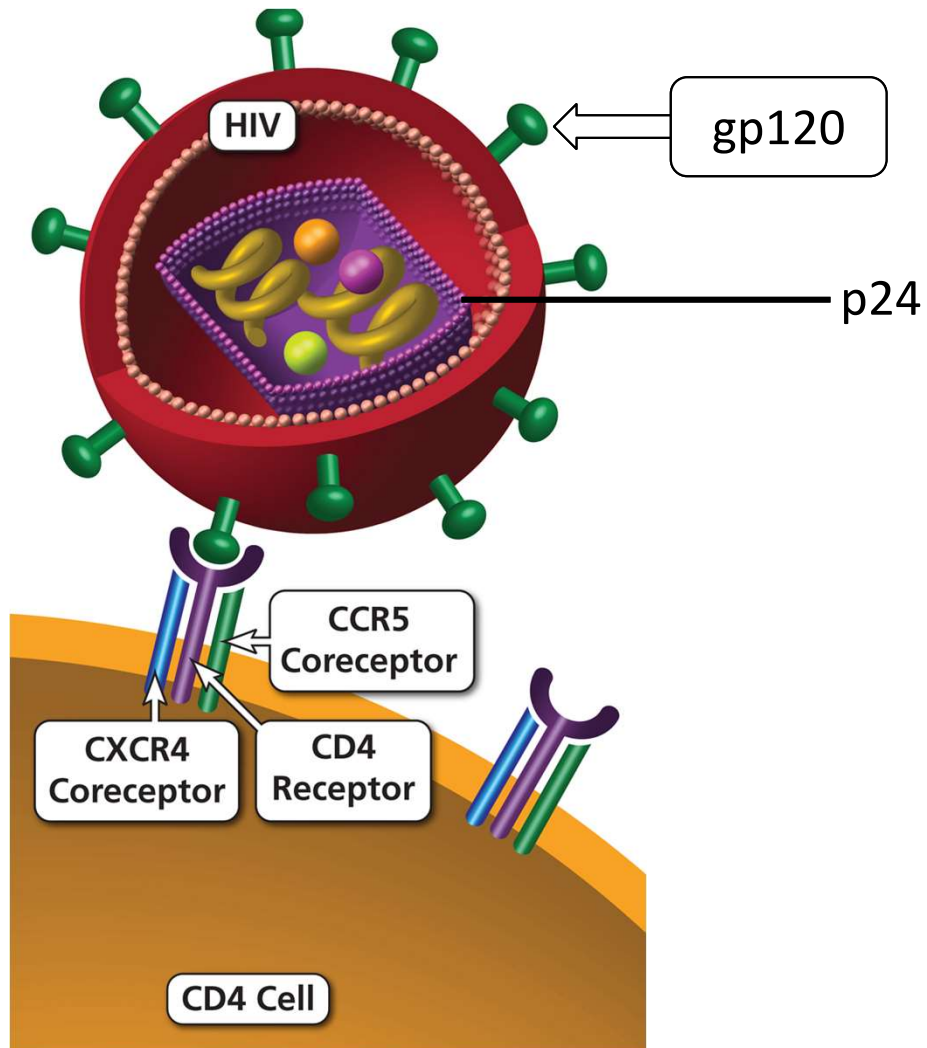


Retrovirus Structure

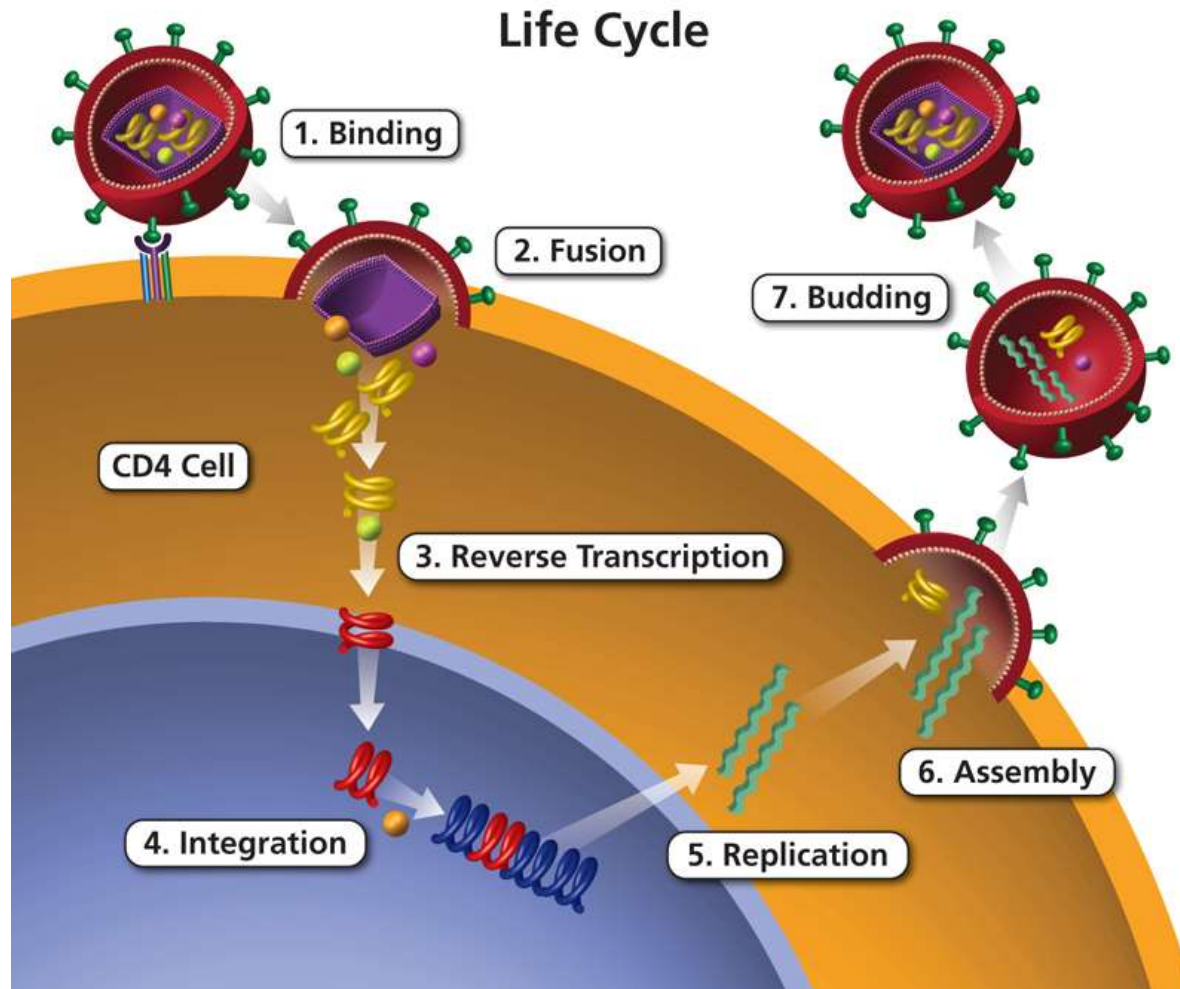
Retrovirus Replication Cycle



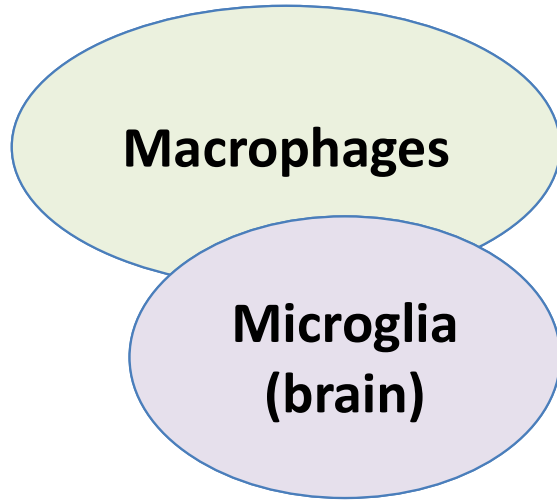
Human Immunodeficiency Virus



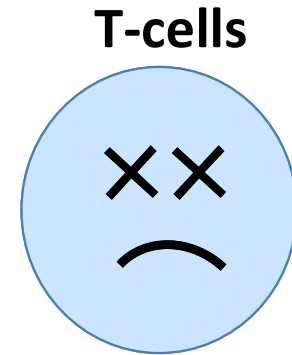
Human Immunodeficiency Virus



Effects of HIV on human cells

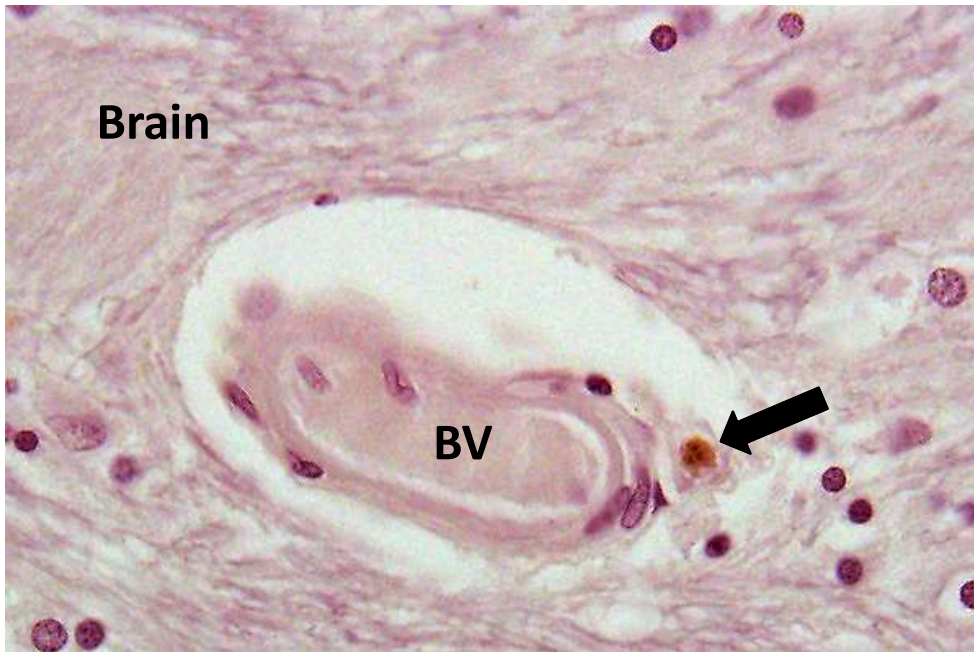



- Non-lytic infection
- Productive making more virus
- Latent but can be triggered to replicate



- Lytic infection
- Immunodeficiency

People Living with HIV



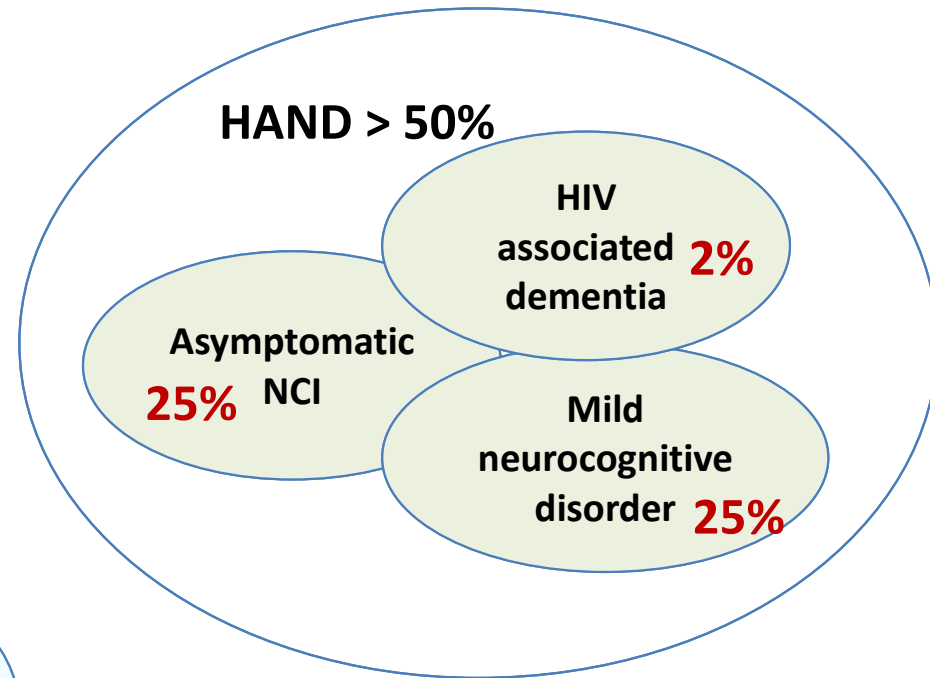
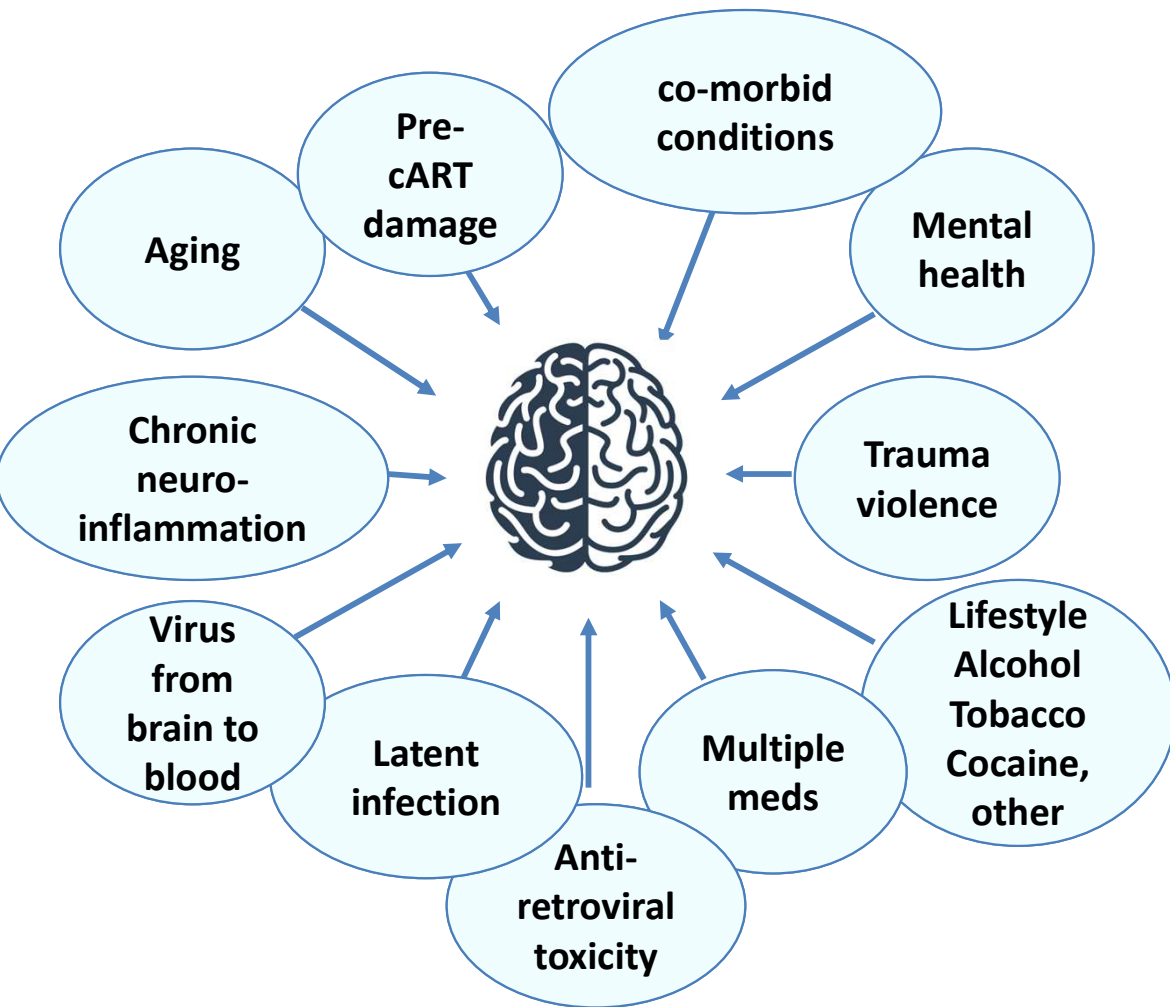
 p24+ viral particle crossing the blood brain barrier



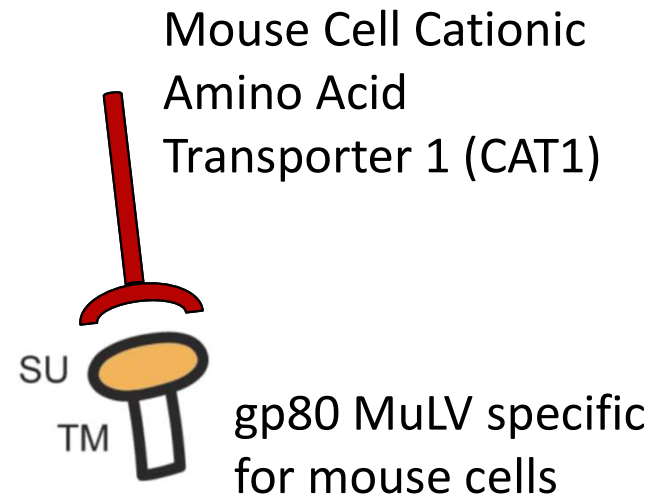
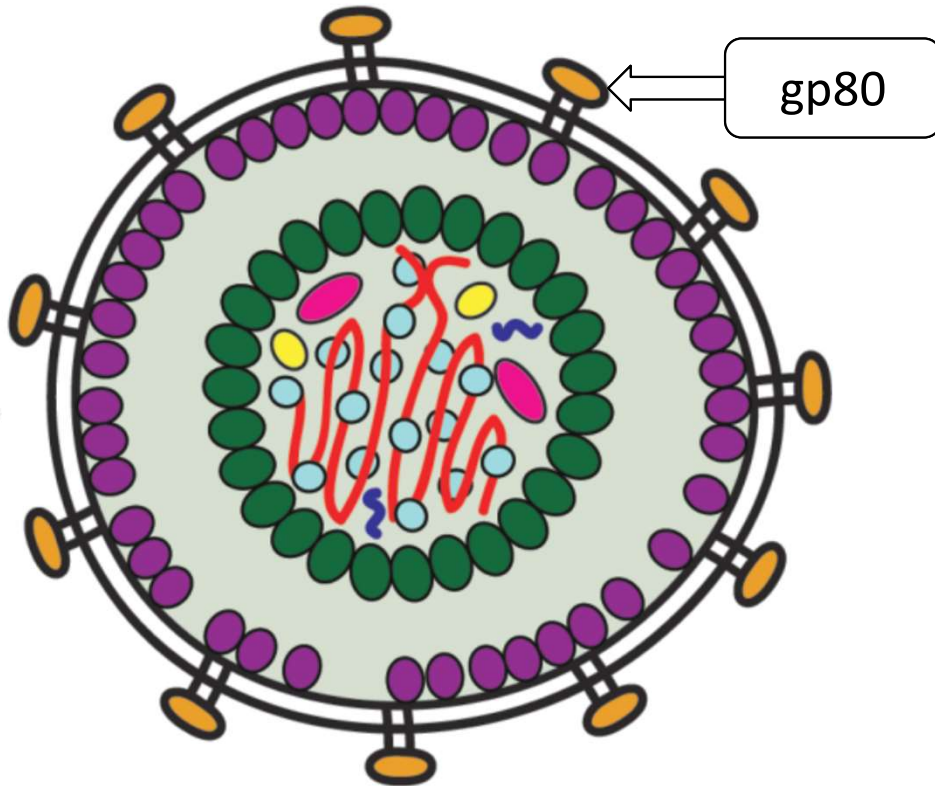
p24 ELISA to track viral infection in humans from blood sample

People Living with HIV

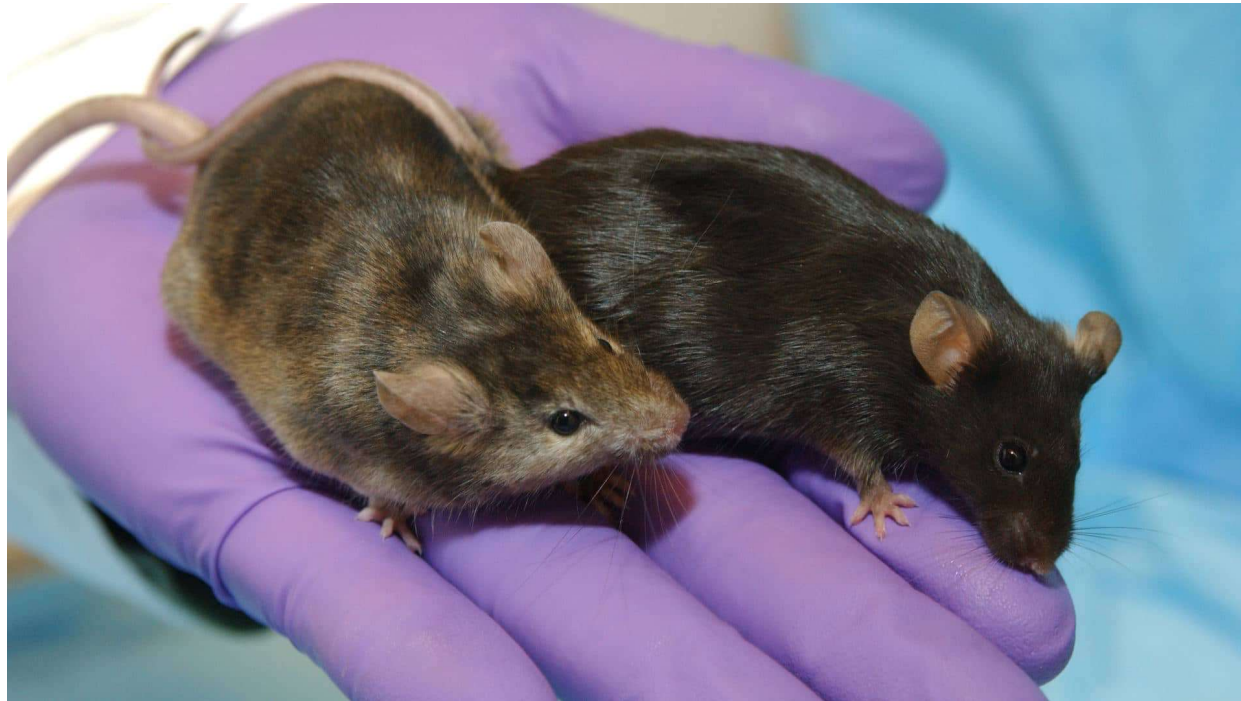
HIV Associated Neurocognitive Disorder (HAND)



Ecotropic Murine Leukemia Virus

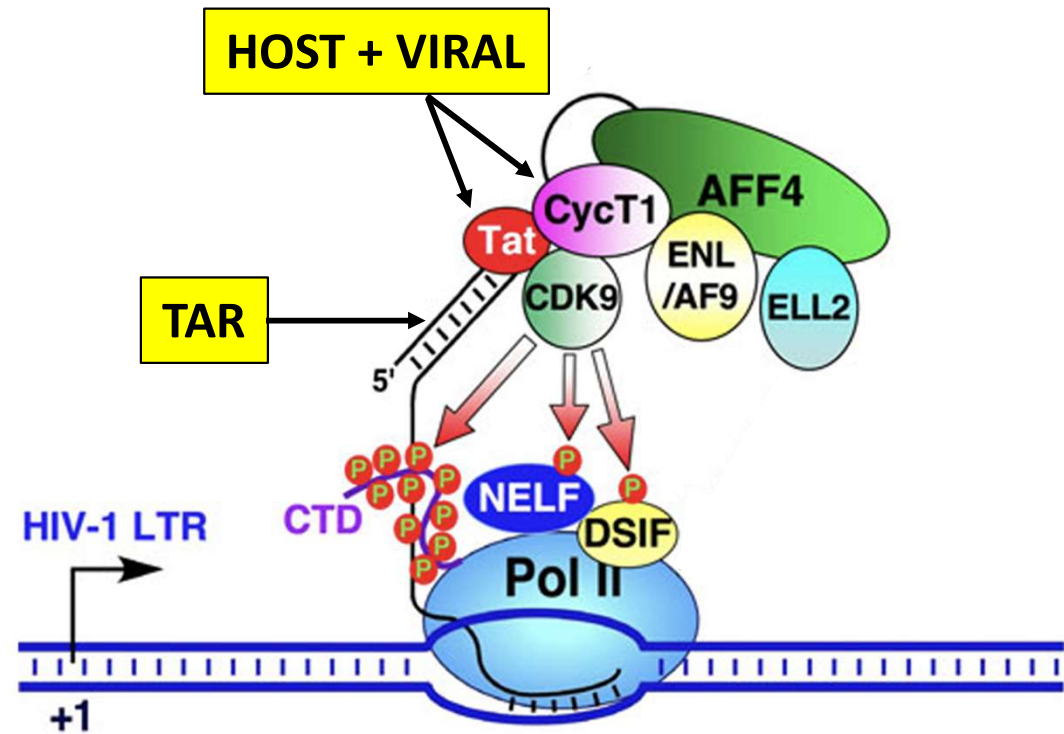


Effects of MuLV in mice



little to no known effects

Human Immunodeficiency Virus



- Following reverse transcription and viral DNA integration into host cell genome, an RNA loop is formed called **TAR**.
- The early viral protein **Tat** binds to **TAR** and recruits several **host proteins** to the transcription factor complex to promote transcription of viral DNA into RNA.
- Viral particles are assembled and released from infected cells.

Barriers to HIV infection of mouse cells

Viral entry

gp120 vs gp80

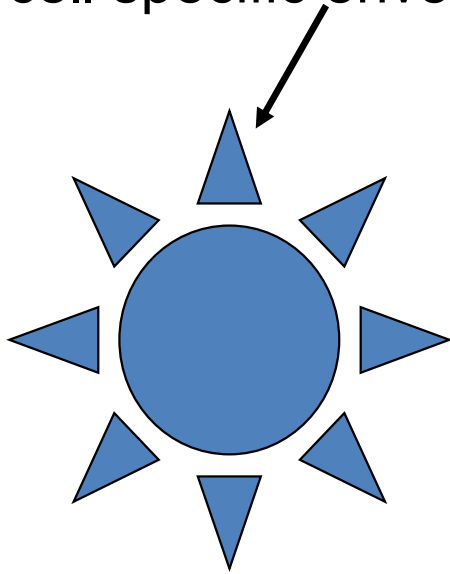
**Transcription
Factor Complex**

Tat and mouse cyclin T1

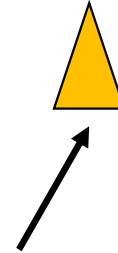
What are the hazards?

Two horizontal lines are positioned below the text. The top line is light purple and spans most of the width of the slide. The bottom line is light green and is shorter, starting further to the right and extending to the right edge of the slide.

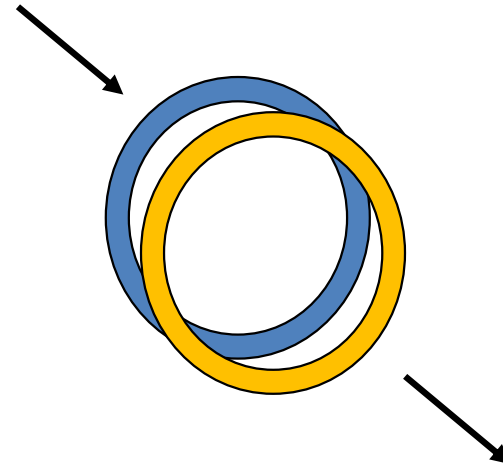
Human cell specific envelope proteins

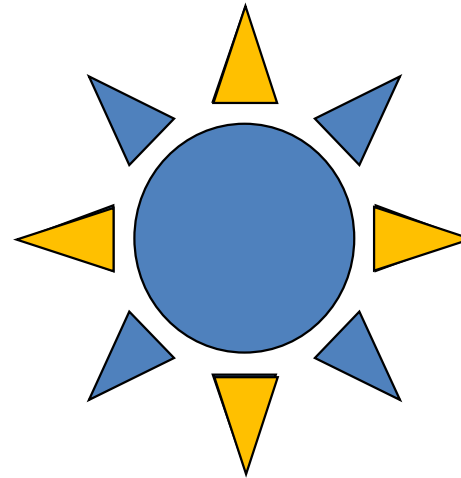
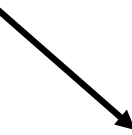
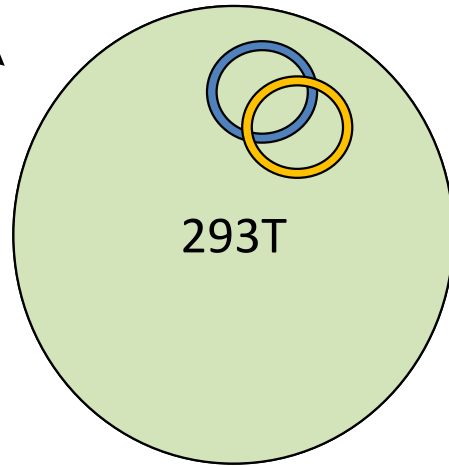
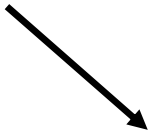
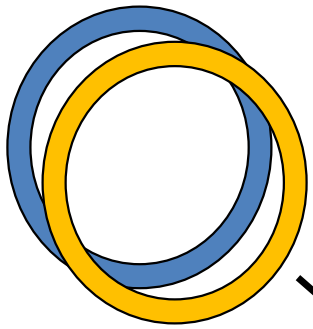


Mouse cell specific glycoprotein
(15ug plasmid DNA into 293T cells)



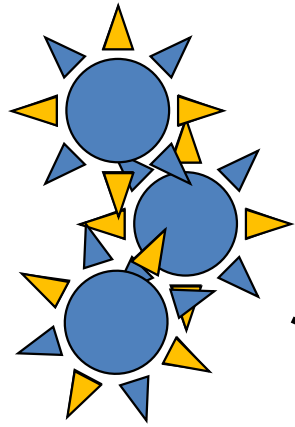
Plasmid vector driven HIV production
via pYU2 or pYK-JRCSF
(10ug plasmid DNA into 1.5×10^6 293T cells)



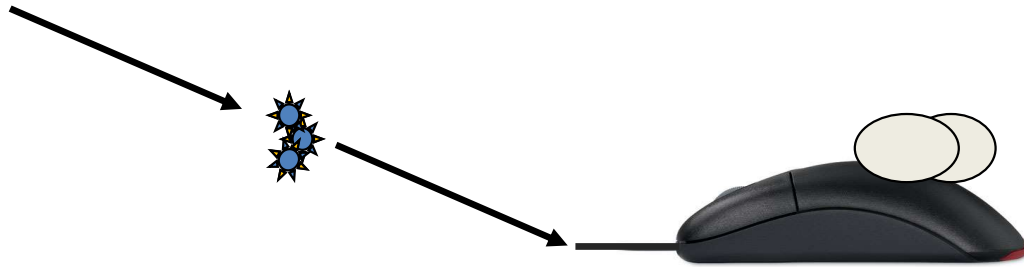


Cell permissive for transfection
of human or mouse cells

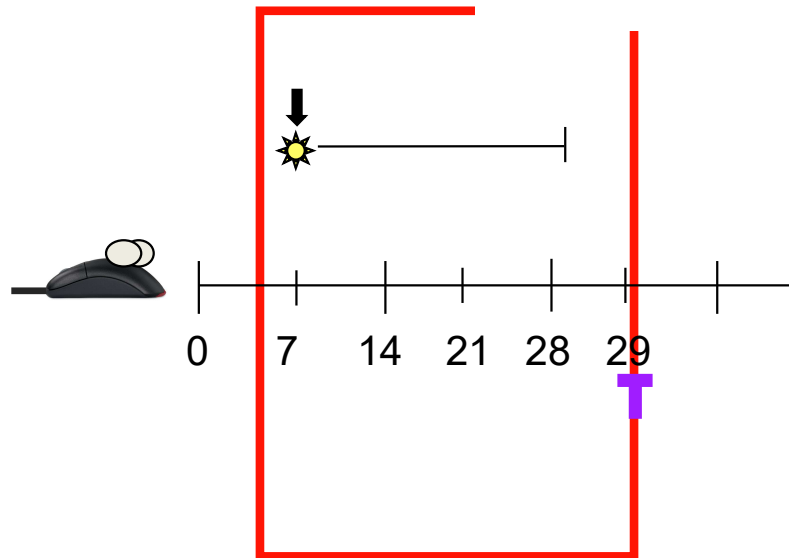
Pseudotyped virus



Cell free supernatants with 10^4 - 10^5 pg p24 in 0.1ml saline



Pseudotyped virus



Window of risk =22 days

WHO IS AT RISK?

- Everyone who comes in contact with mice

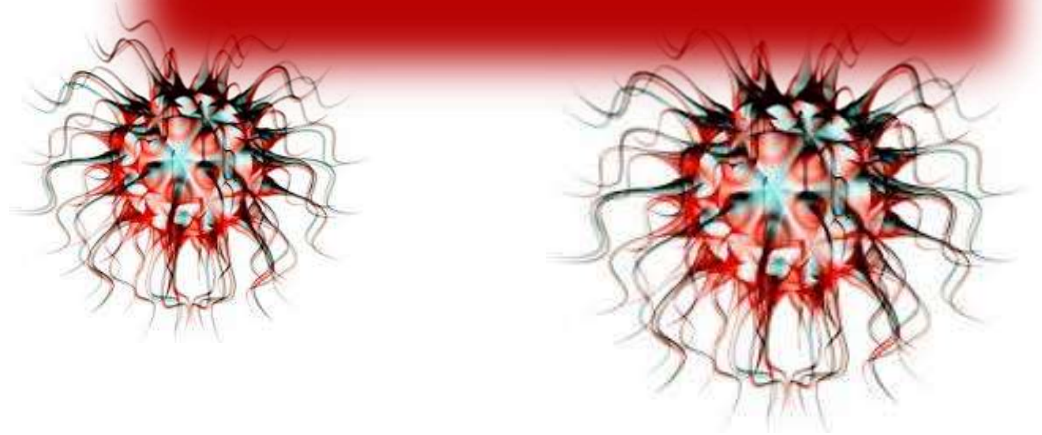
HOW TO MANAGE PROJECT?

- Restricted access
- Have trained personnel change bedding/food /water
- Provide enhanced PPE for those in contact with mice

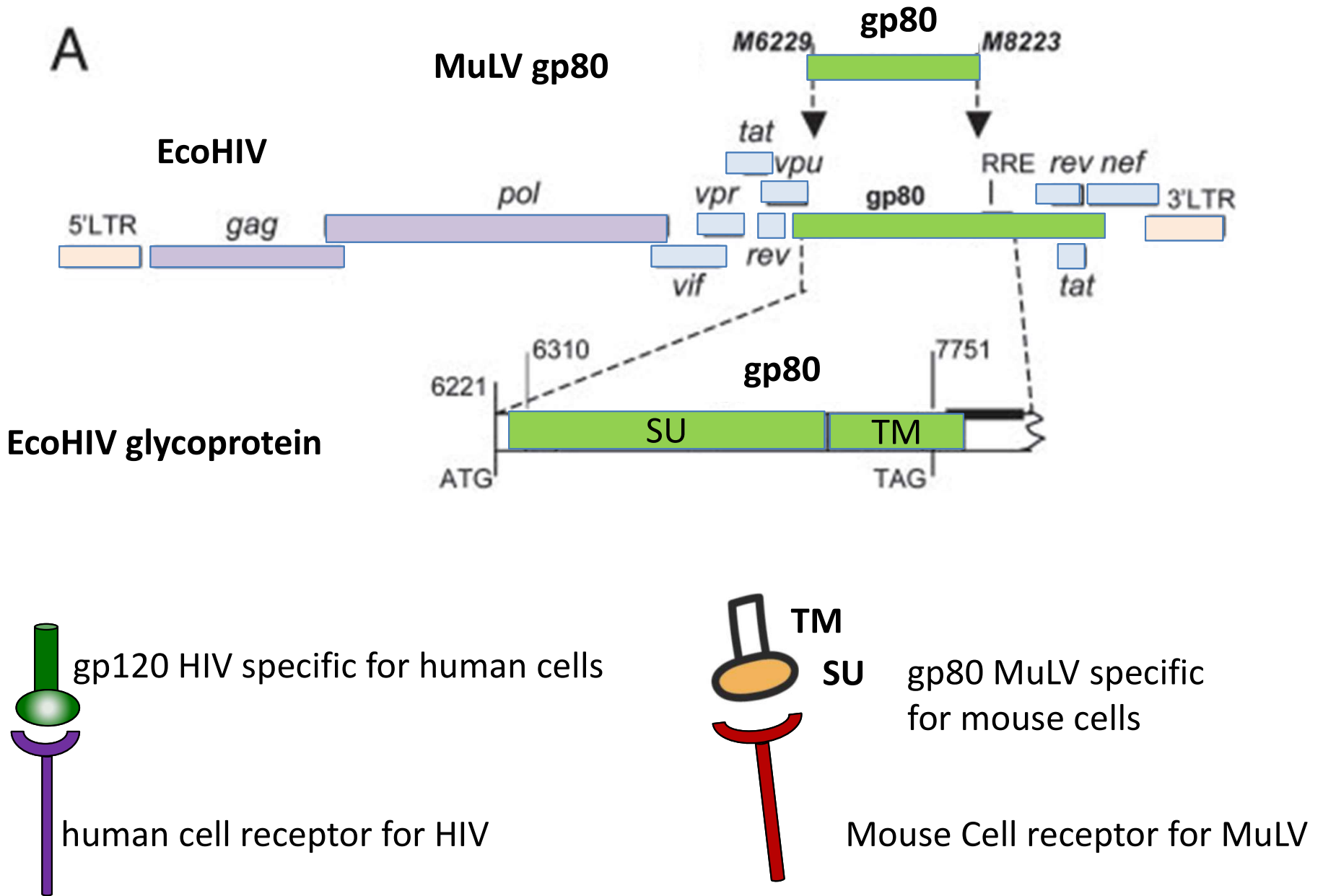
GENES OUT



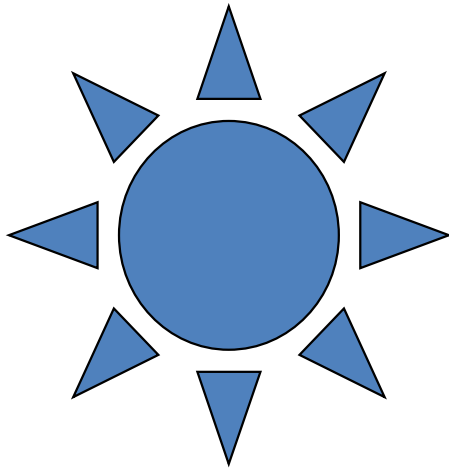
VIRUS IN



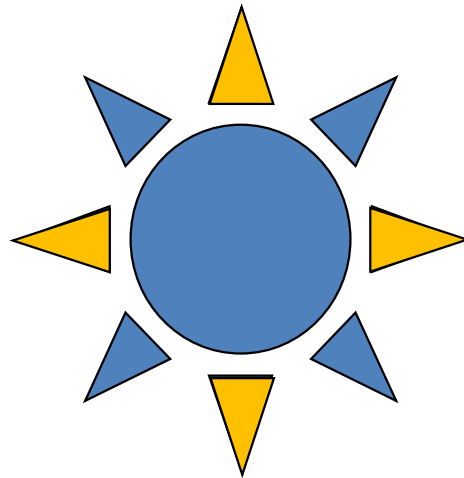
A



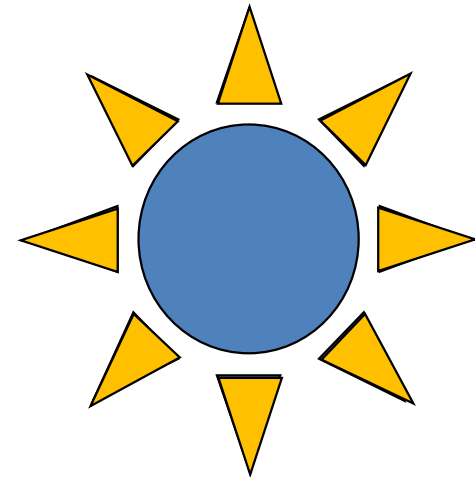
Pseudotyped HIV in the mouse model



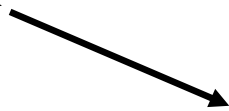
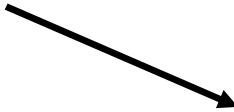
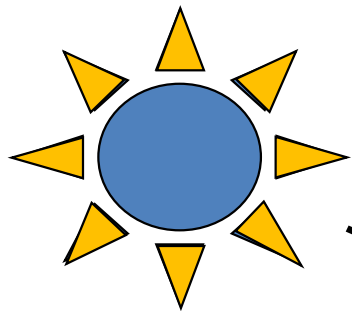
HIV- HUMAN



HIV- HUMAN/MOUSE



EcoHIV- MOUSE



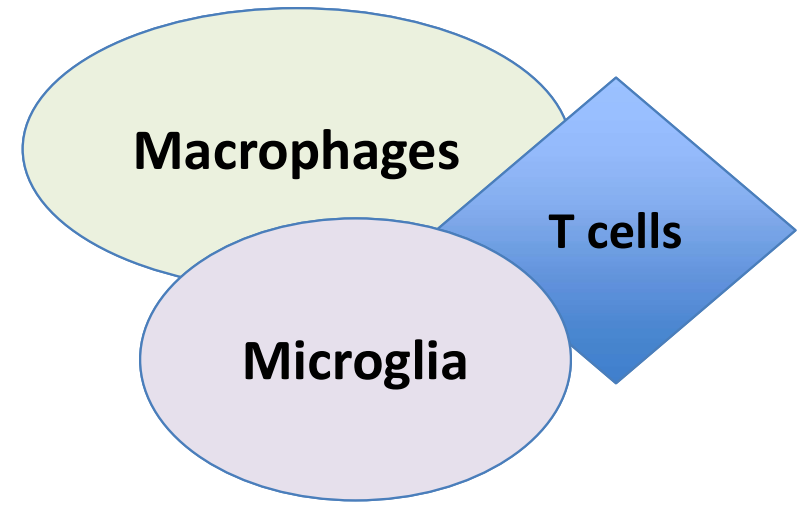
4.0×10^6 pg p24 in 250ul per mouse, IP



HIV gp120 is replaced by ecotropic murine leukemia virus envelop, gp80, but otherwise contains all HIV genes.

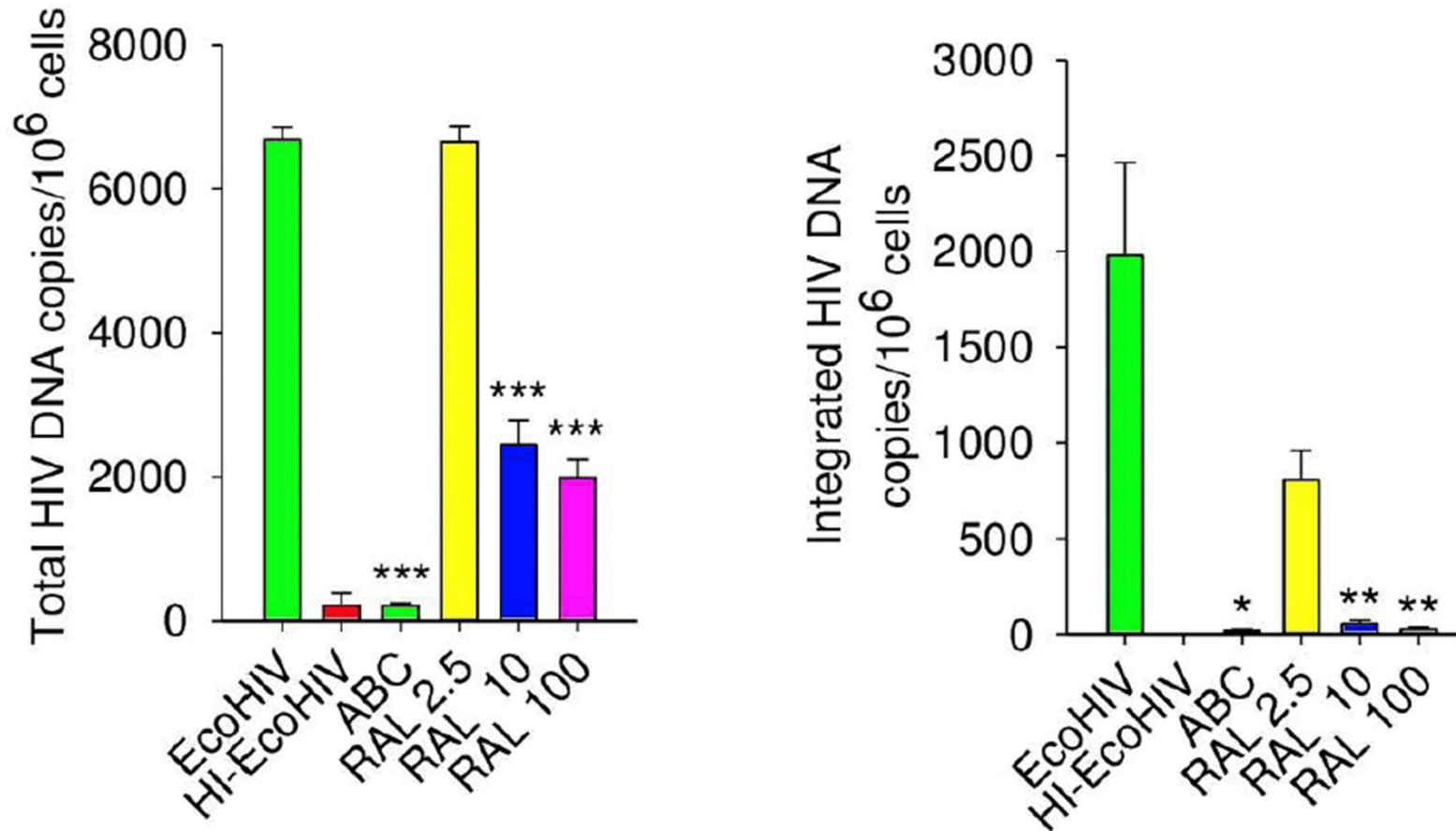


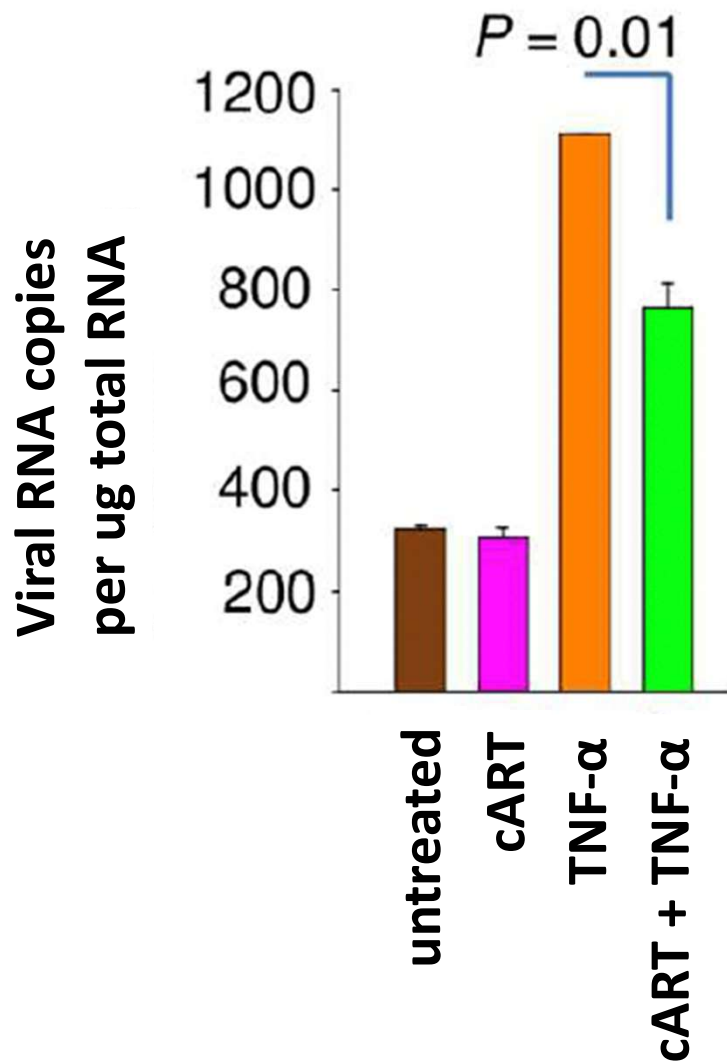
p24 ELISA to track viral infection in mice



- Initial burst of replication
- Adaptive immune response limits replication
- Chronic neuroinflammation
- Blood brain barrier disruption
- Low levels of macrophage and microglial infection
- Virus can become integrated into host DNA, but remain latent until triggered to replicate

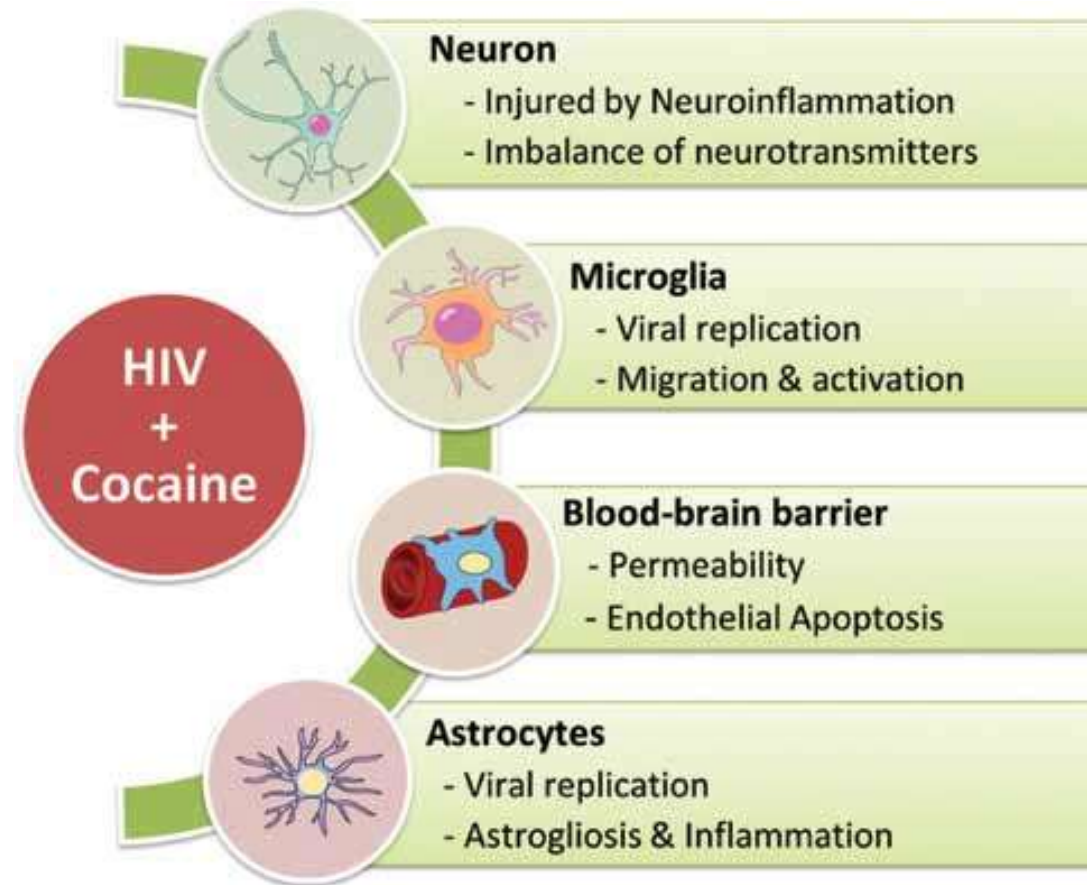
EcoHIV integrates efficiently in the murine host genome






- **EcoHIV in macrophage reservoirs (latent) is transmissible.**
- **cART suppresses viral replication.**
- **Latent virus can be activated by TNF-α even in presence.**

People with HIV who use Cocaine



People with HIV who use Cocaine


 **NIH Public Access**
Author Manuscript
J Neuroimmune Pharmacol. Author manuscript; available in PMC 2012 December 1.

Published in final edited form as:
J Neuroimmune Pharmacol. 2011 December ; 6(4): 503–515. doi:10.1007/s11481-011-9297-0.

Cocaine and HIV-1 Interplay: Molecular Mechanisms of Action and Addiction

Shilpa Buch^{1,*}, Honghong Yao¹, Minglei Guo², Tomohisa Mori³, Tsung-Ping Su³, and John Wang²

NIH-PA Author Manuscript

REVIEW ARTICLE
Front. Microbiol., 08 September 2015 | <https://doi.org/10.3389/fmicb.2015.00931> 


Interactive effects of cocaine on HIV infection: implication in HIV-associated neurocognitive disorder and neuroAIDS

SCIENCE
OPEN

OVER 3500 articles brain, HIV and cocaine

Received: 13 June 2016
Accepted: 27 July 2016
Published: 18 August 2016

Epigenetic Remodeling
Thangavel Samikkannu, Venkata S. R. Atluri & Madhavan P. N.

 **NIH Public Access**
Author Manuscript
Curr HIV Res. Author manuscript; available in PMC 2013 November 12.

Published in final edited form as:
Curr HIV Res. 2012 July ; 10(5): 425–428.

Cocaine and HIV-1 Interplay in CNS: Cellular and Molecular Mechanisms

Shilpa Buch^{1,*}, Honghong Yao¹, Minglei Guo², Tomohisa Mori³, Blaise Mathias-Costa¹, Vijeta Singh¹, Pankaj Seth⁴, John Wang², and Tsung-Ping Su³

NIH-PA Author Manuscript

People with HIV who use Cocaine

- Cocaine increases rate of HIV replication
- Alters astrocyte and neurons metabolism
- Increases cognitive impairments in PWH
- Increases cytokine production in he brain

Cocaine Use Compounds HIV-Related Memory Loss, Study Finds

Abstaining from cocaine use can help HIV-positive people with cognition

Wednesday, January 22, 2020

Investigator(s): [Matthew Wright, PhD](#)

Research Theme(s): [Neurotherapeutics](#)



What's next? Using EcoHIV mice to account for all viral components (except gp120)

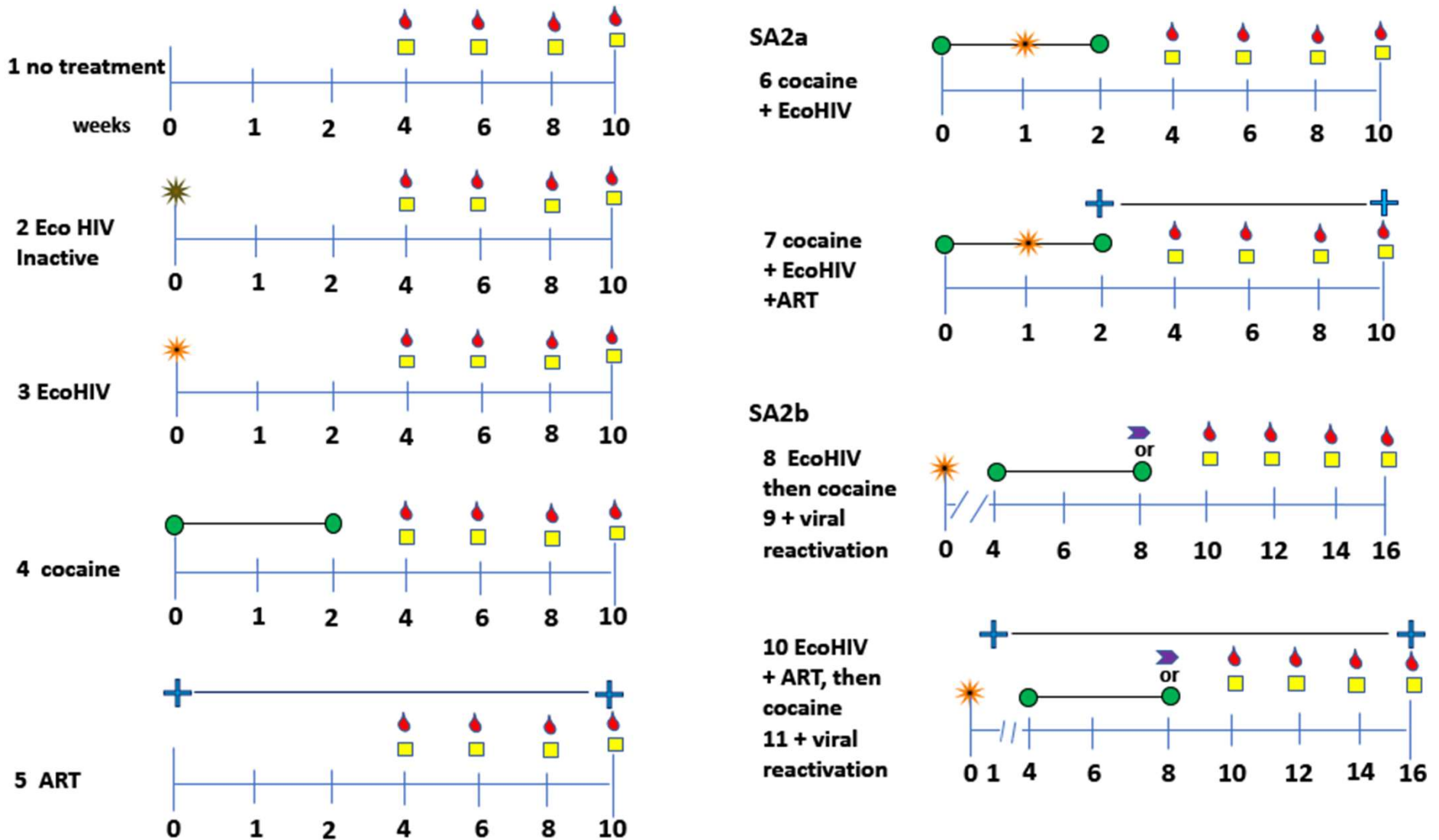


Figure 11. Schematic of AIM 2.

■ Euthanasia,
 🔥 Blood,
 ★ Virus,
 ● Cocaine,
 + ART,
 ➡ Reactivation: SAHA, prostratin

Safety First

TOHAZAR

