

# T-Pharmacytes for the Targeted Eradication of HIV Reservoirs

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## Viral Reservoirs as a Barrier to HIV Eradication

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- Antiretroviral therapy (ART) suppresses HIV viremia to undetectable levels but fails to eradicate infection

Why?

- Infection is established in long-lived memory CD4+ T-cells – half-life = 44 weeks

# Flush and Kill Approach to HIV Eradication

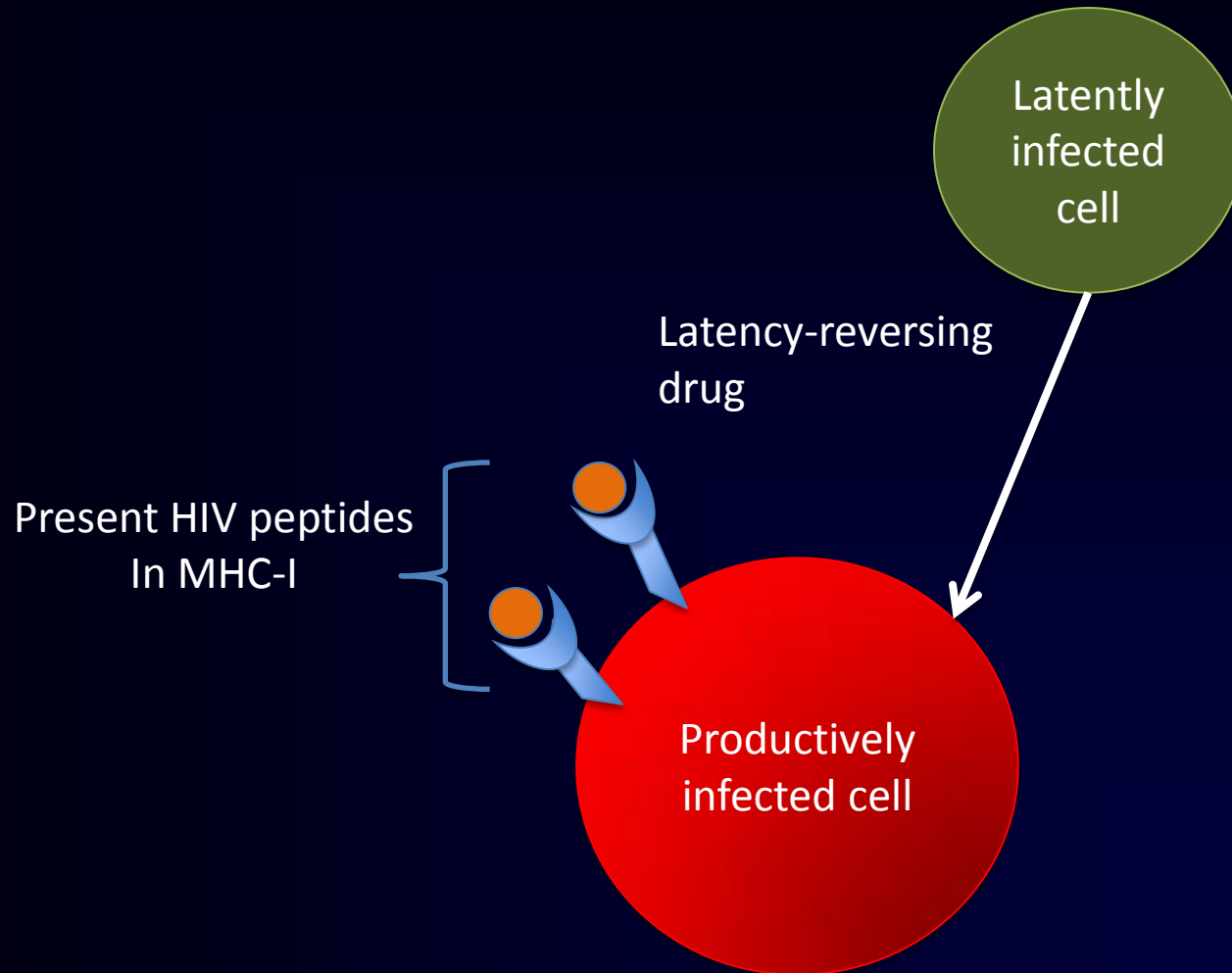
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Latently  
infected  
cell

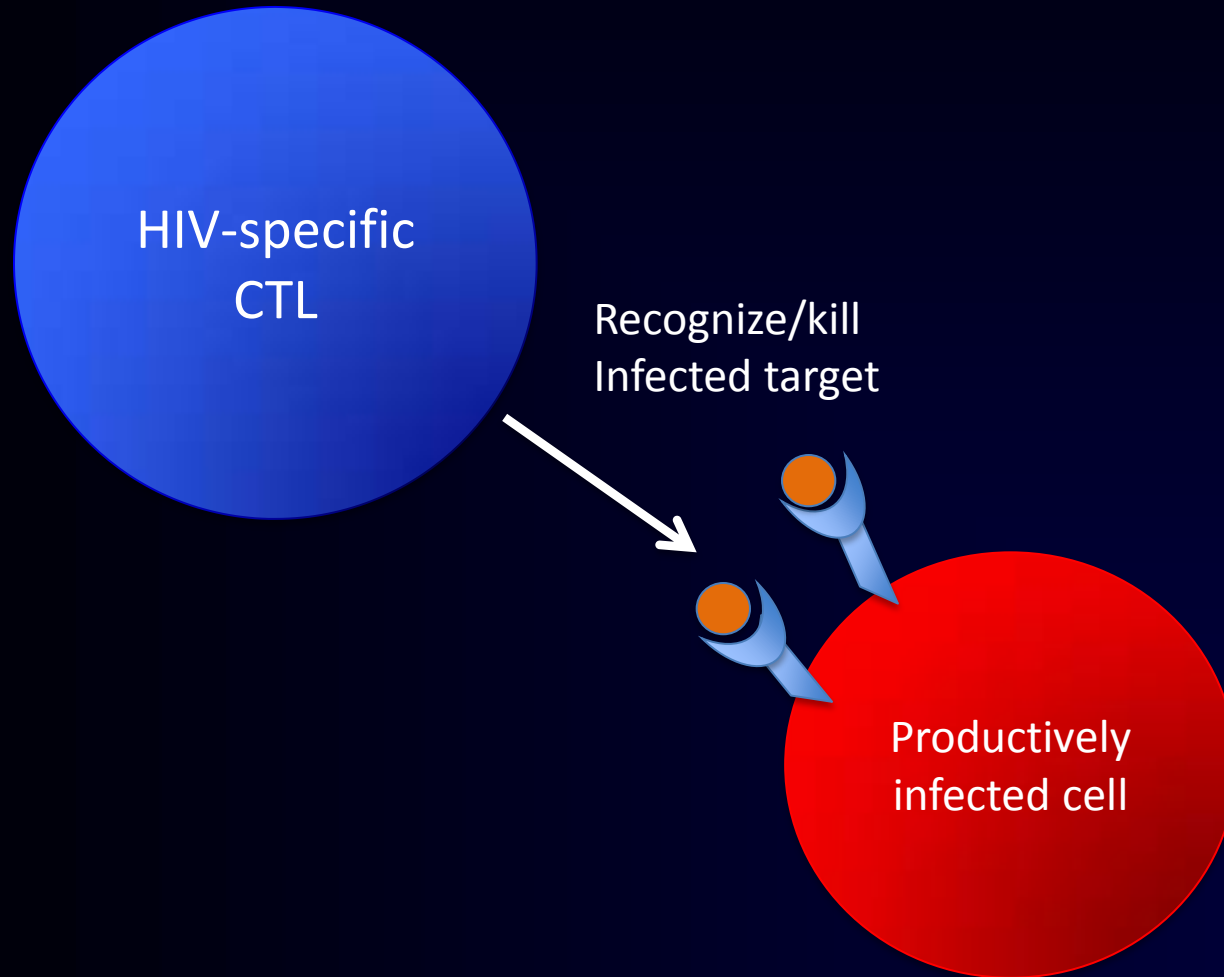
# Flush and Kill Approach to HIV Eradication

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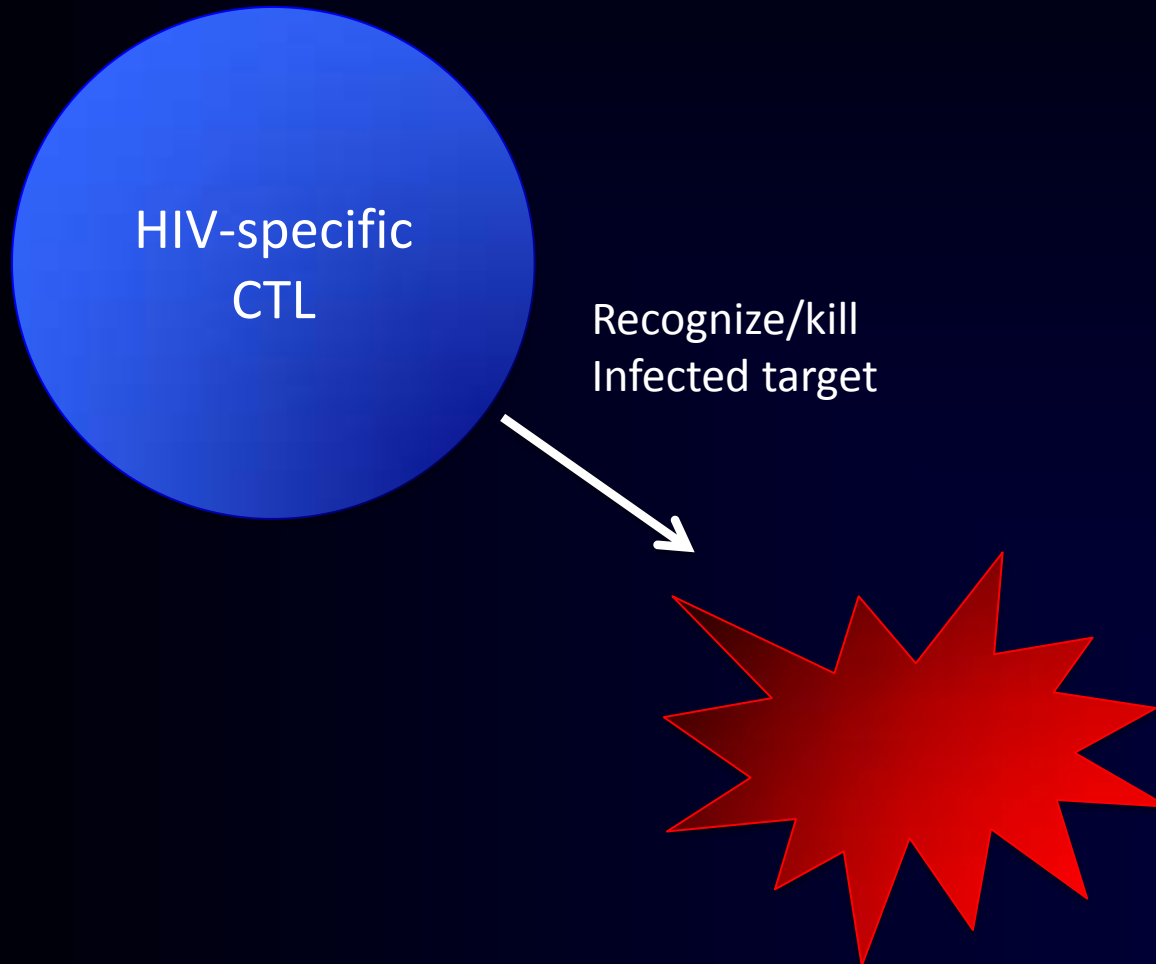
# Flush and Kill Approach to HIV Eradication

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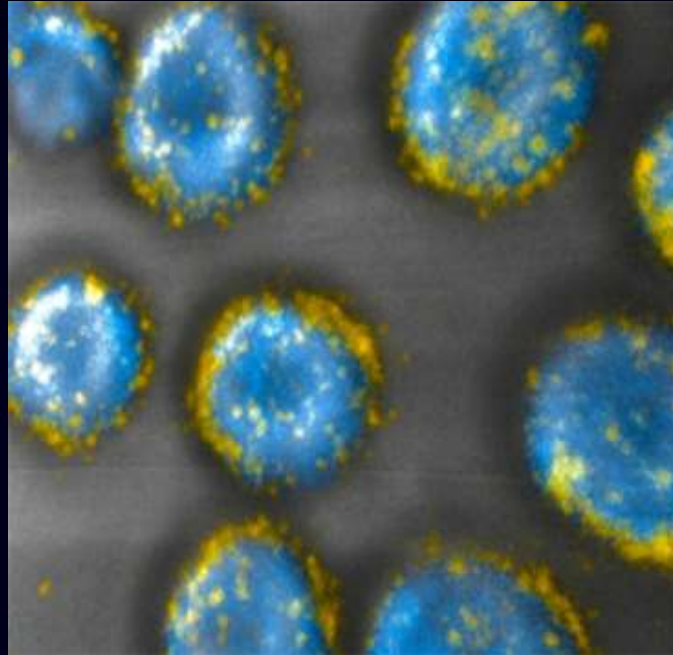
# Flush and Kill Approach to HIV Eradication

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## 'T-Pharmacytes' – CTL conjugated to drug-loaded nanoparticles

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- Conjugate HIV-specific CTL to nanoparticles loaded with latency-reversing drugs
- T-Pharmacytes given by adoptive immunotherapy



Mathias Stephan



## Characterization of T-Pharmacytes

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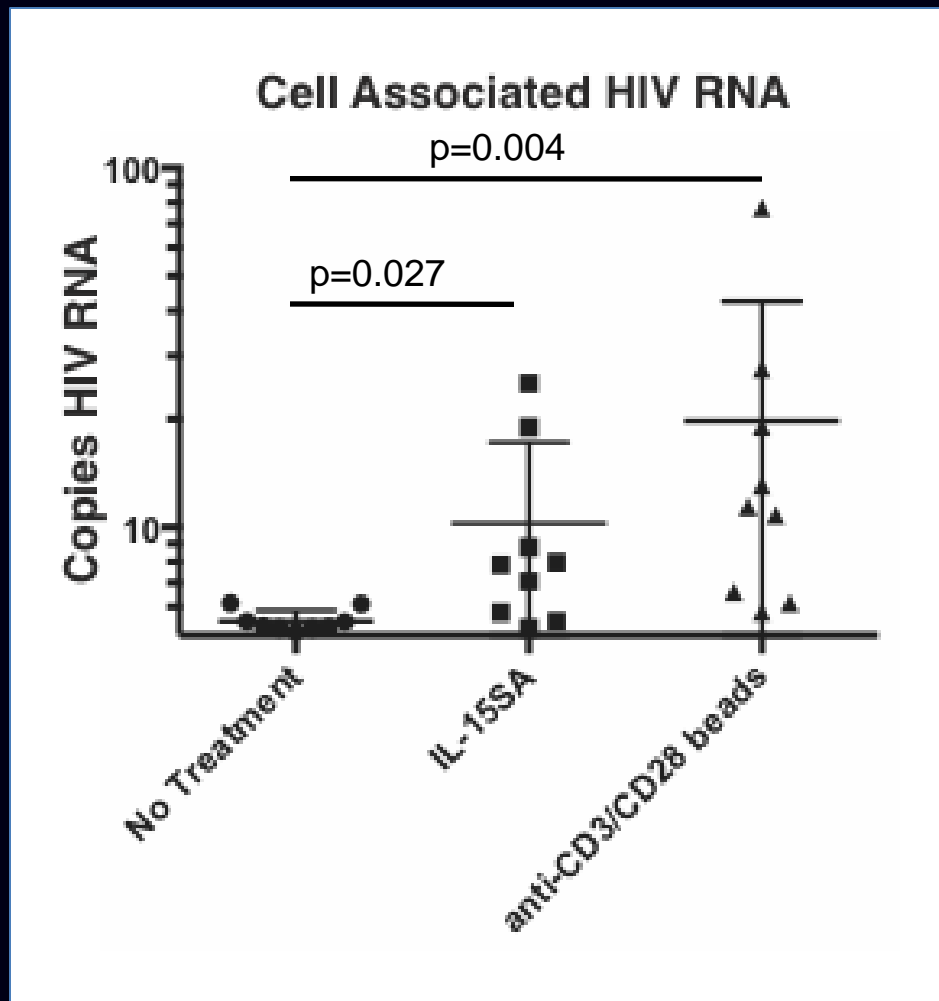
- Developed to provide cytokine support to adoptively-transferred CTL (nanoparticles loaded with IL-15 superagonist – IL-15SA)

### T-Pharmacytes:

- Traffic normally and accumulate at sites of antigen expression in murine tumor models
- Exhibit unimpaired killing of target cells *in vitro*
- Expand and persist *in vivo* dramatically better than normal CTL (when loaded with IL-15SA)
- Eradicate established melanoma tumors where normal CTL failed

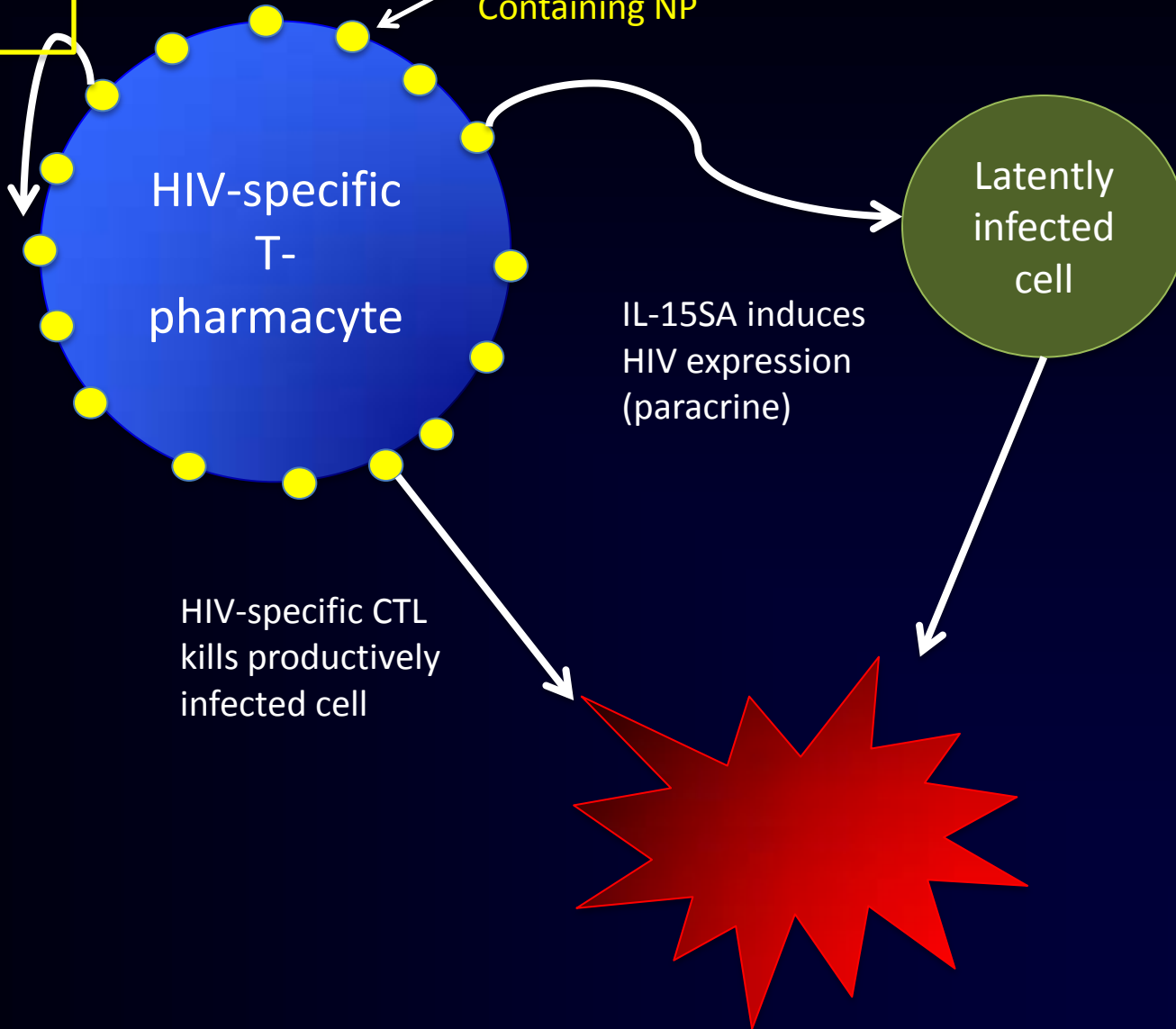
# IL-15SA Reverses HIV Latency from Patient Samples

- Purified resting CD4+ T-cells treated with IL-15SA or anti-CD3/CD28 beads



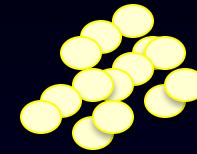
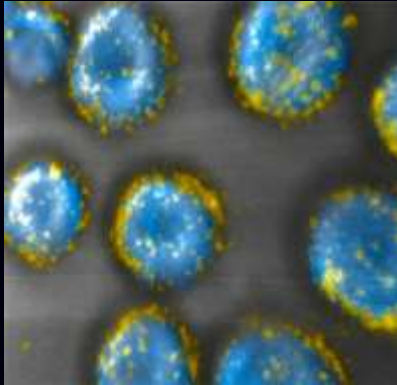
IL-15SA enhances survival/function (autocrine)

IL-15SA Containing NP



# Latency Reversal by T-Pharmacytes

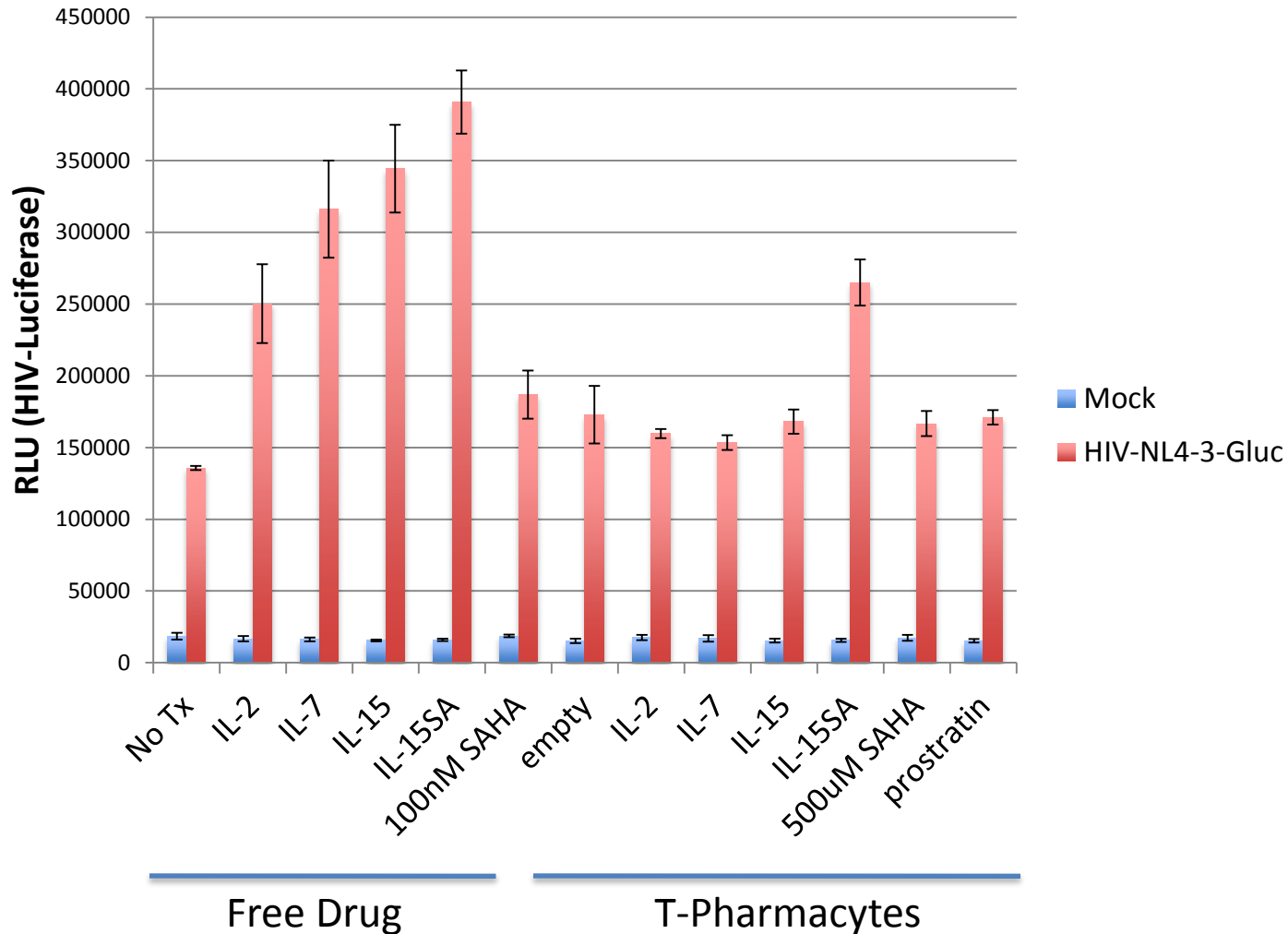
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Resting primary CD4+ T-cells  
Infected with luciferase HIV

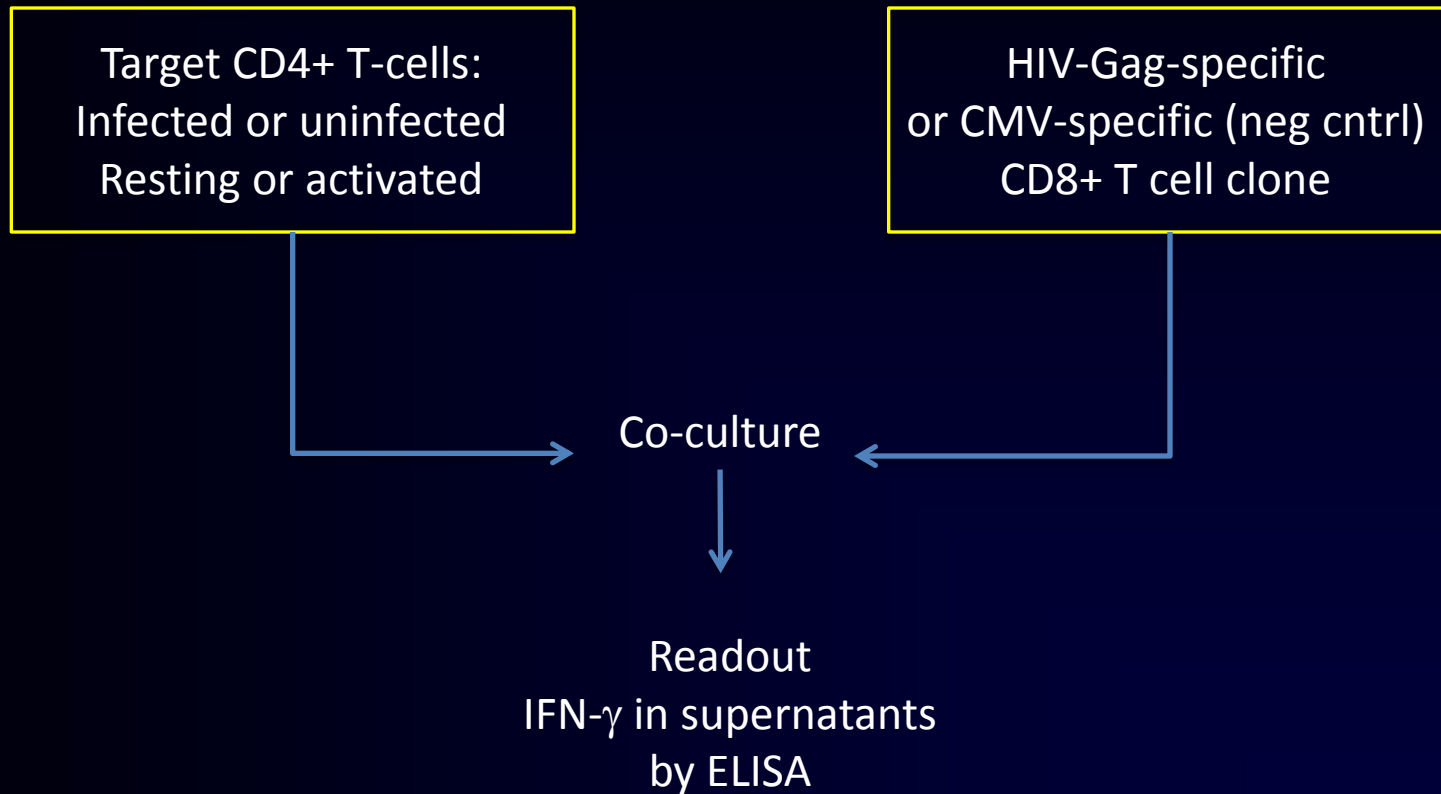


# IL-15SA-loaded T-Pharmacytes Reverse HIV Latency



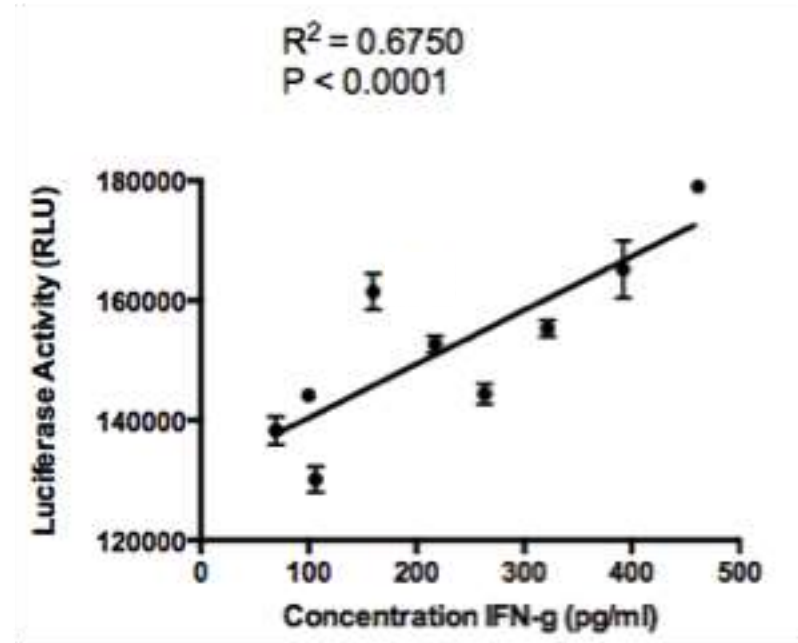
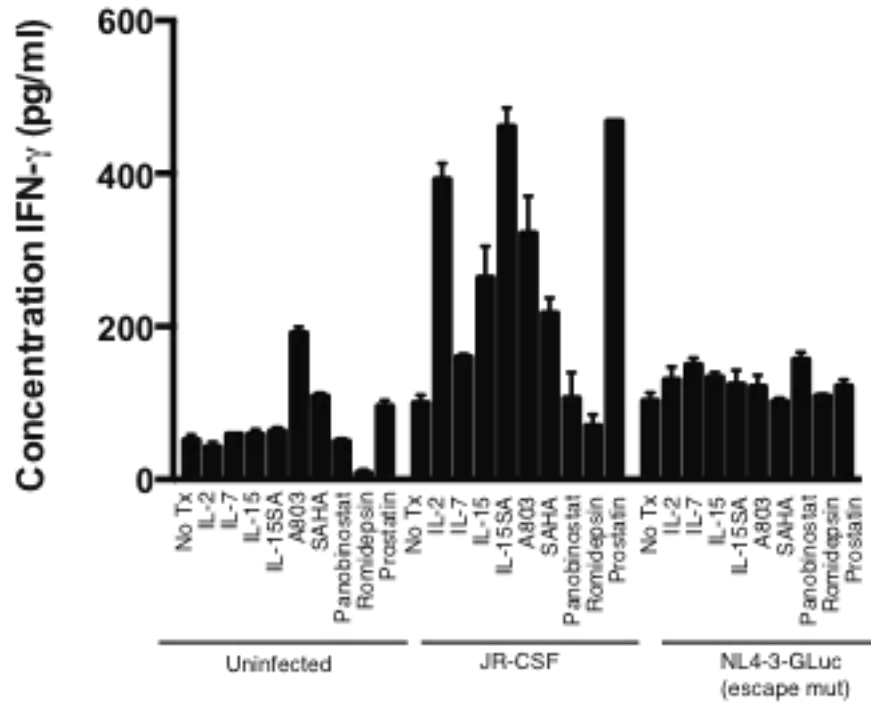
# CTL Reservoir Recognition Assay

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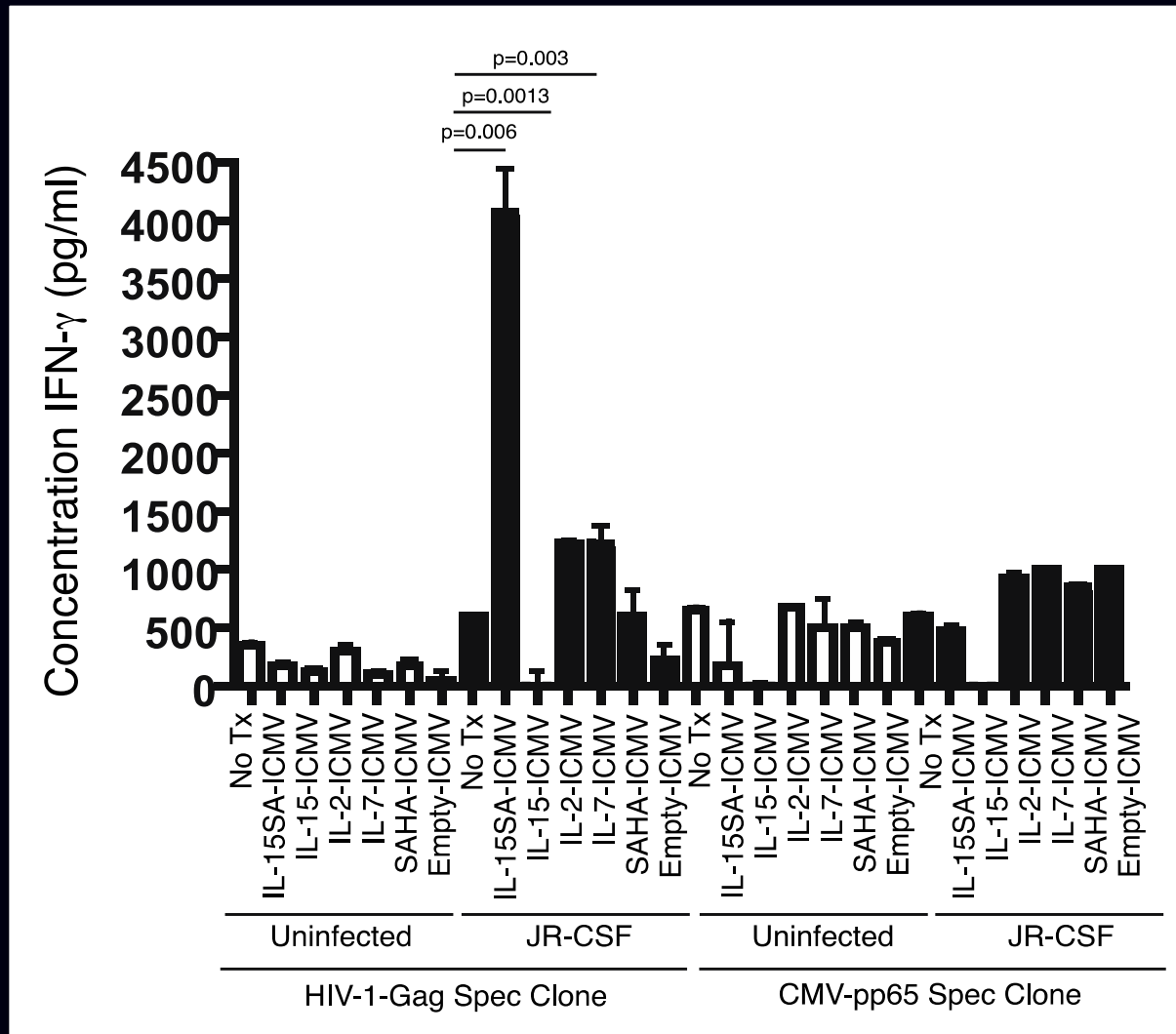


# Latency Reversal Recognition Assay (drugs washed out)

## OM9 SL9



# Day 7 co-culture T-Pharmacocytes with latently-infected targets



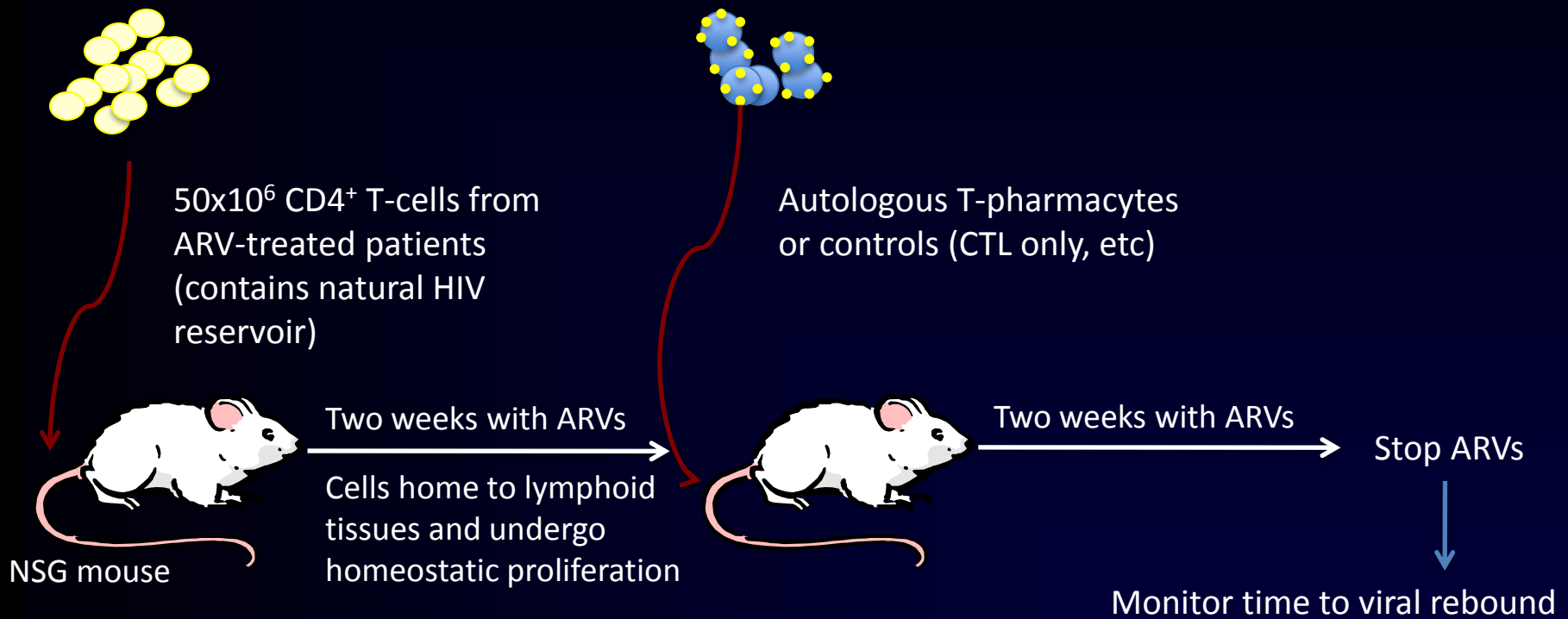


## Testing T-Pharmacyte Eradication Strategy in Humanized Mouse Model

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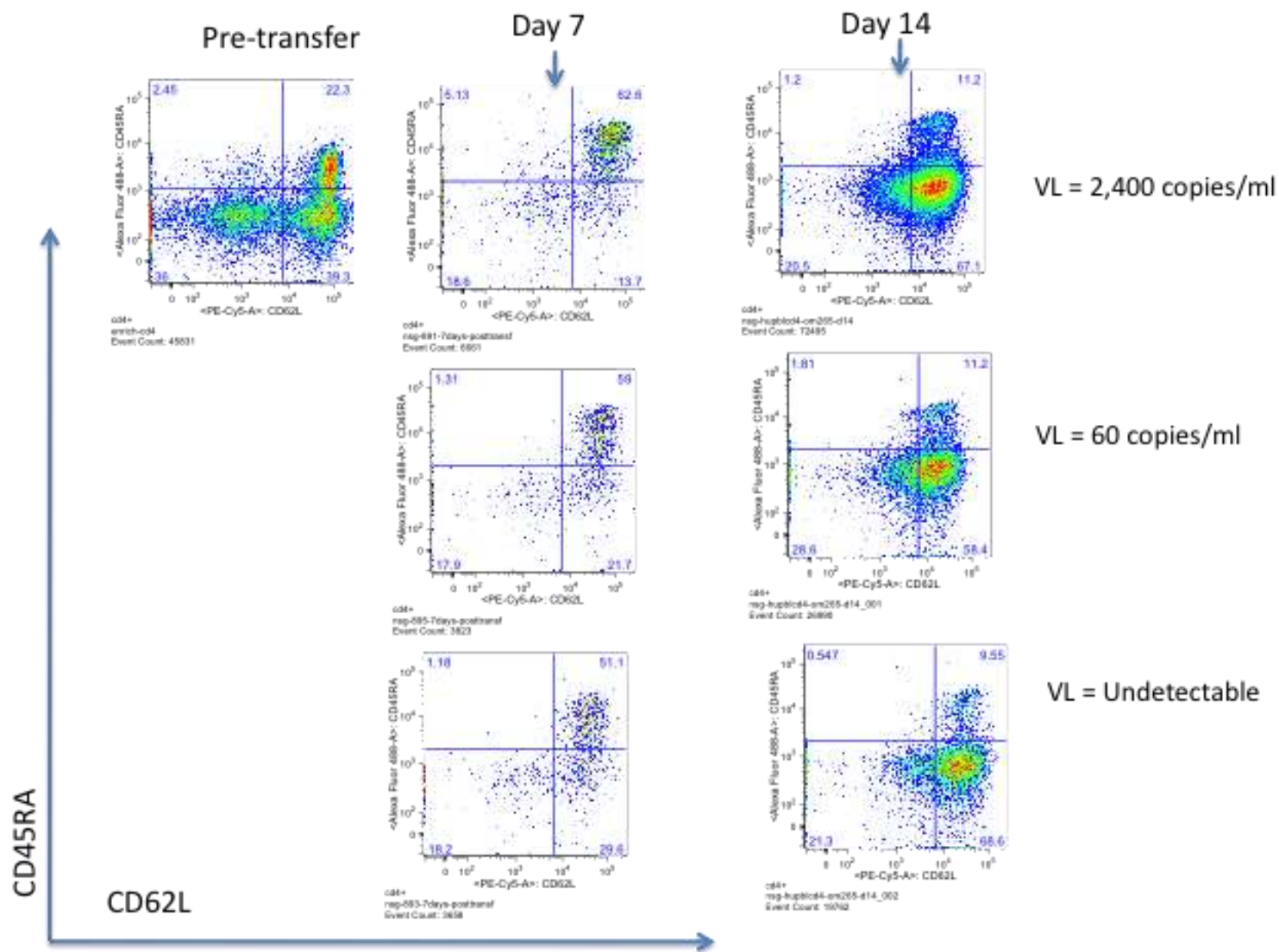
- Long durations of treatment are required to establish latent reservoirs *in vivo*
- Problematic for non-human primate studies due to high cost
- Problematic for existing humanized mouse models as animals progress to graft-versus host disease
- Can we develop a more rapid humanized mouse model?

# huCD4-NSG Model



# huCD4 NSG model

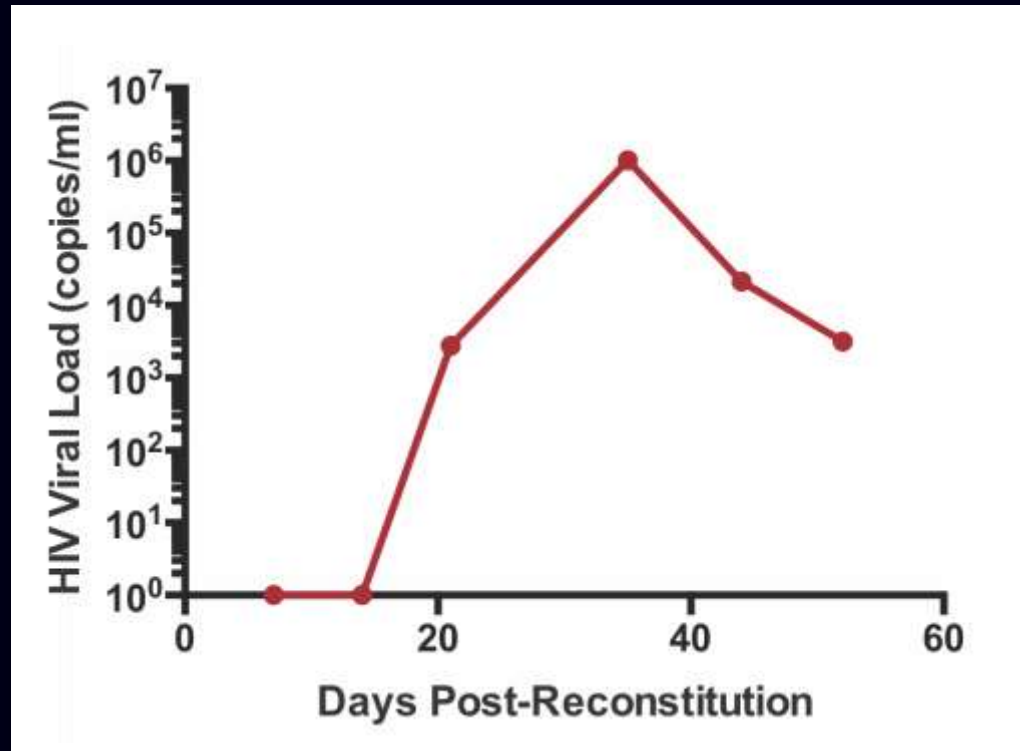
## Maturational States of CD4+ T-cells Following Transfer into NSG Mice



## huCD4 NSG model

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Reconstitute mice with CD4+ T-cells from HIV-infected subject – rebound of endogenous HIV



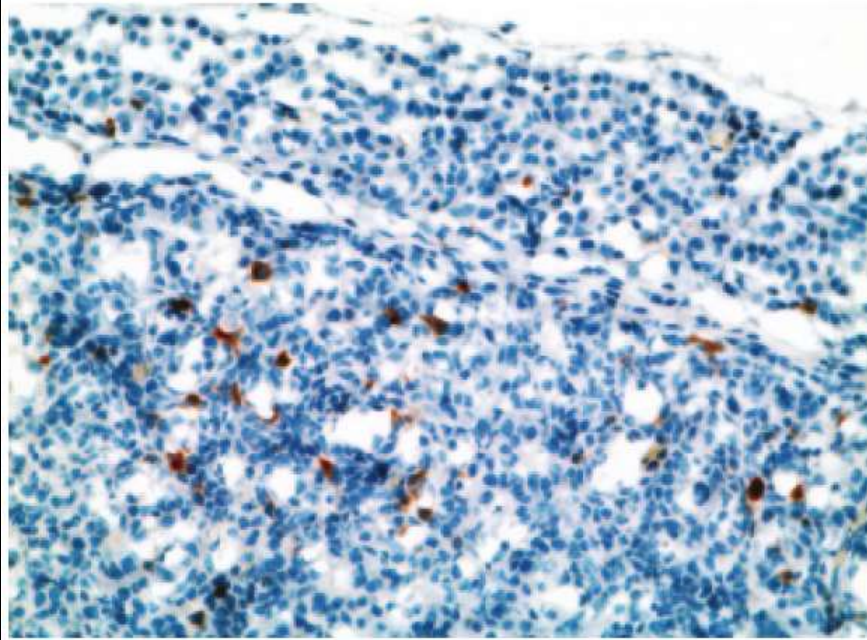
Rebound not observed with cells from 5/6 elite controllers

## huCD4 NSG model

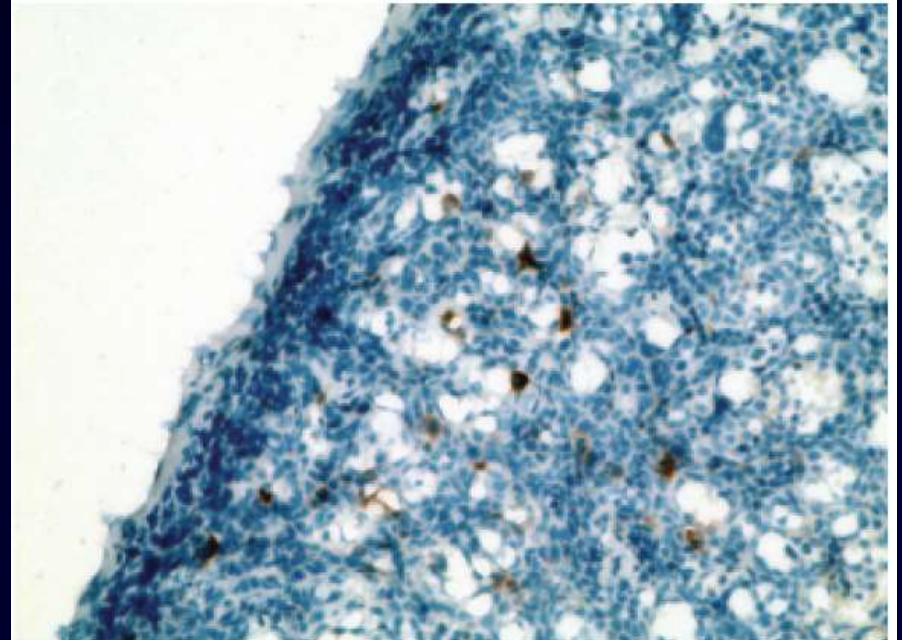
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HIV replication occurs in lymphoid tissue in huCD4 NSG model

Lymph Node



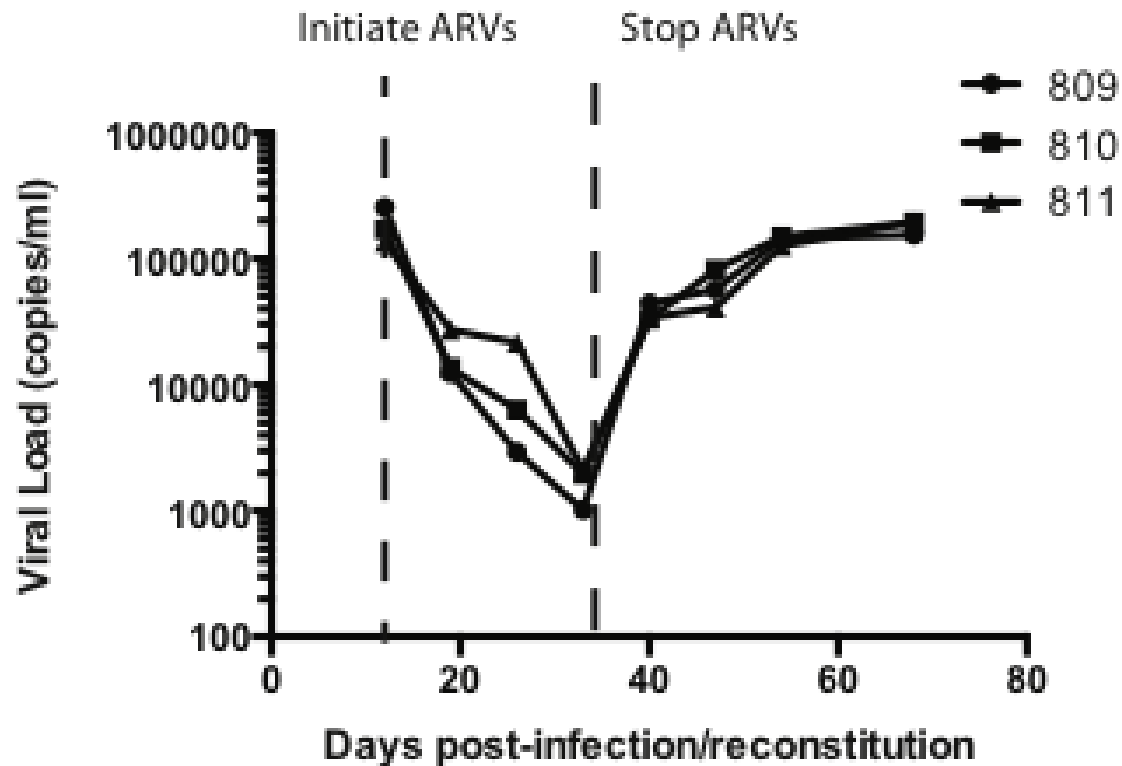
Spleen



Brown – HIV-Gag

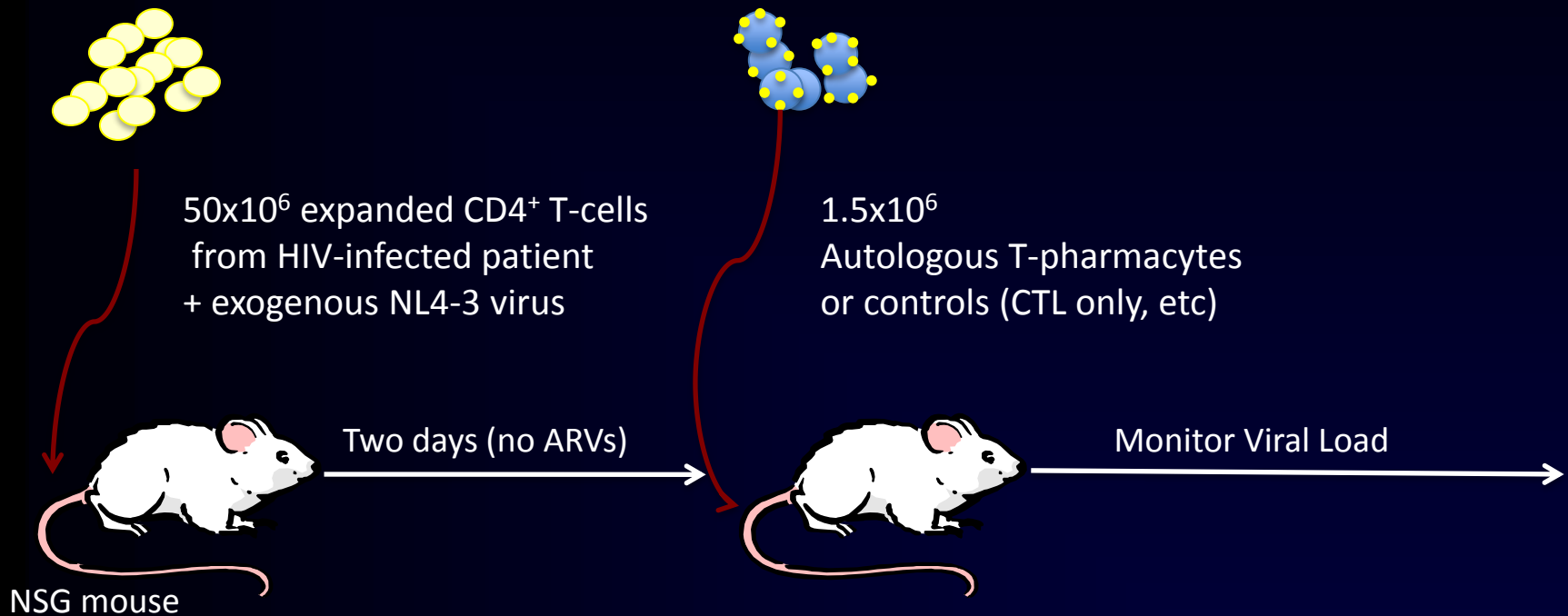
# huCD4 NSG model – ARV Suppression

Pediatric formulations of AZT, 3TC, NVP given in drinking water



# huCD4-NSG – Adoptive Transfer Experiments #1 no ARVs

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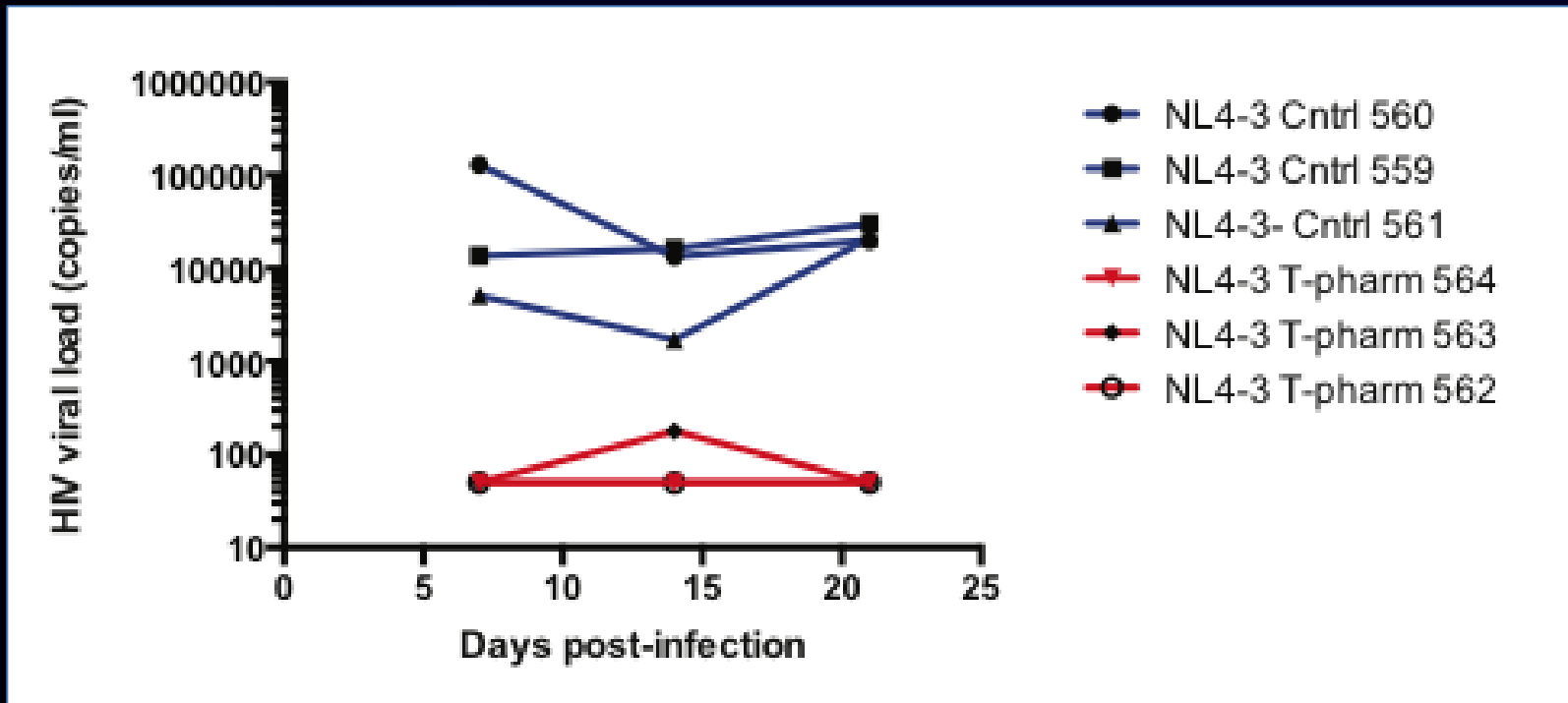


## huCD4-NSG – Adoptive Transfer Experiment #1

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Utilized HIV-Gag-SLYNTVATL-specific CTL clone with potent *in vitro* killing of NL4-3 infected target cells

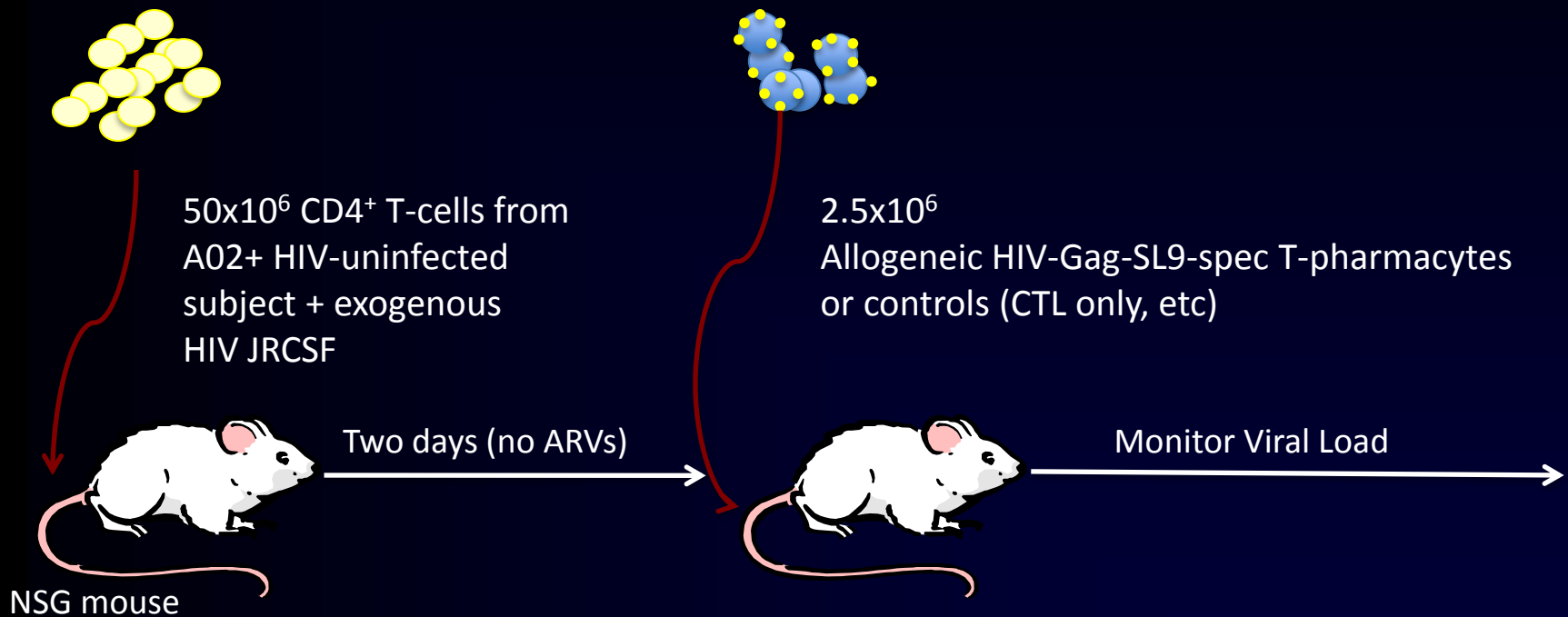
T-Pharmocytes conjugated to nanoparticles loaded with 1.38mg/ml of the IL-15SA ALT-803 (Altor Biosciences)





# huCD4-NSG – Adoptive Transfer Experiments – no ARVs

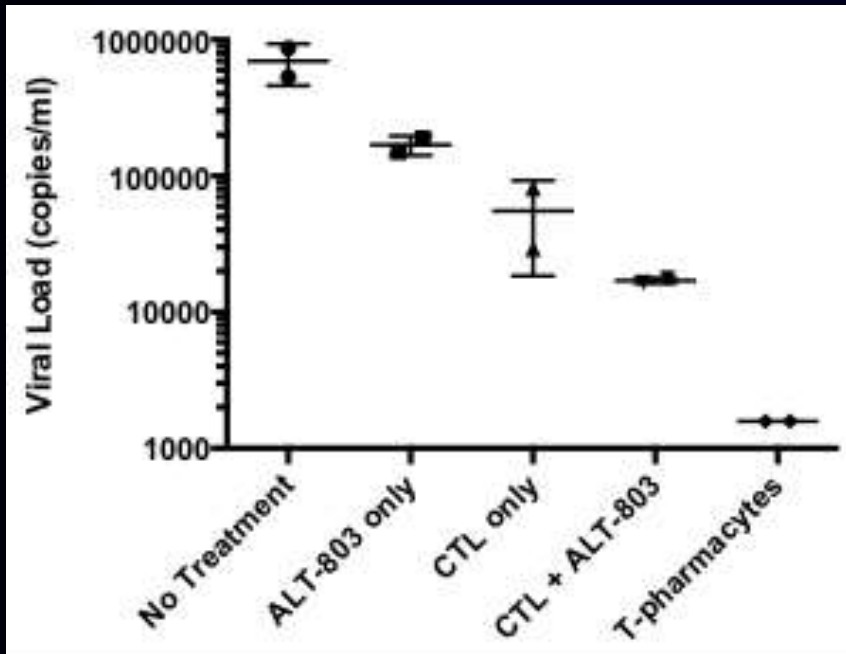
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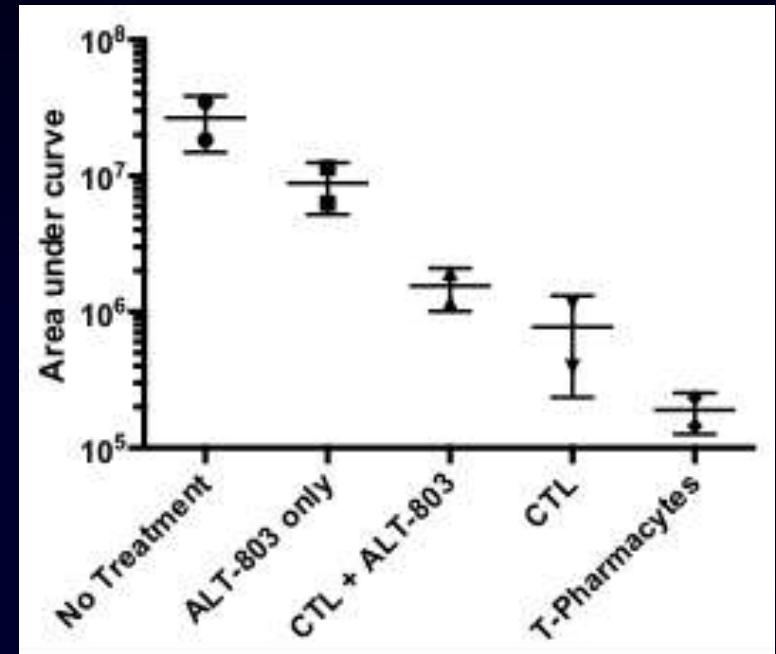


# huCD4-NSG – Adoptive Transfer Experiment #2

Day 17



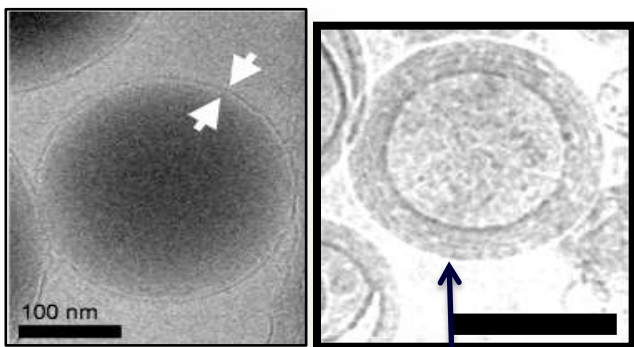
Day 81



## Conclusions

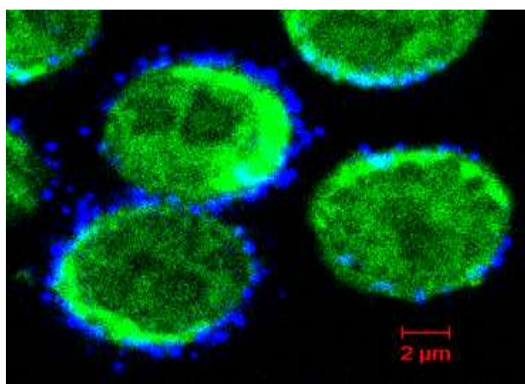
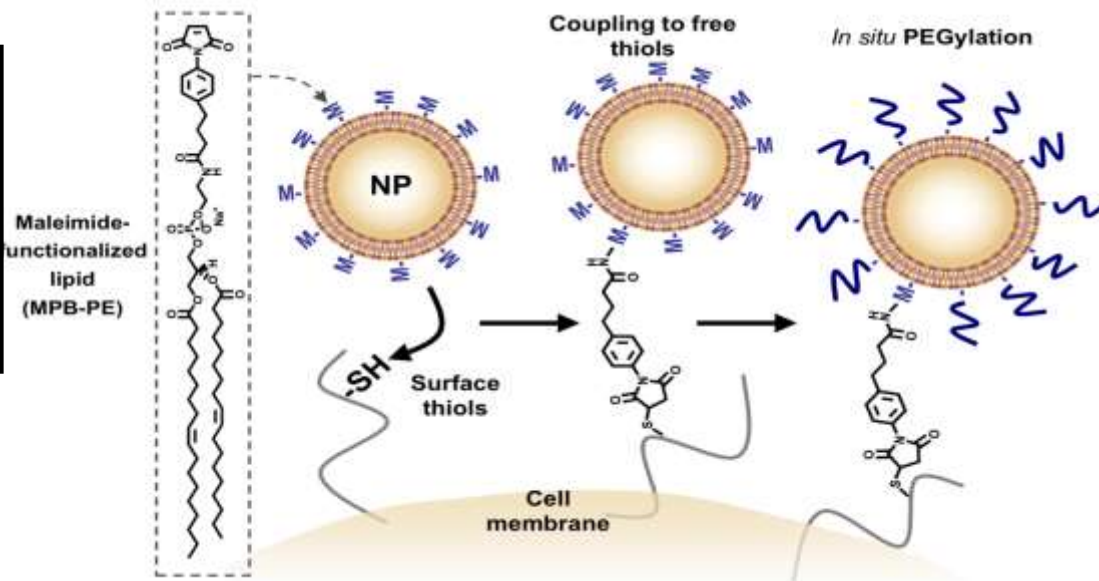
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- T-Pharmacytes carrying the IL-15 superagonist ALT-803 can reverse HIV latency *in vitro* and exert antiviral effects *in vivo*
- The huCD4-NSG mouse is a promising model for eradication studies – including adoptive transfer approaches
- Ongoing studies aim to characterize how the reservoir is shaped by homeostatic proliferation upon transfer into NSG mouse
- Adoptive transfers of T-Pharmacytes into huCD4-NSG mice in the context of ARVs are planned. Does this result in reduction or eradication of reservoirs?

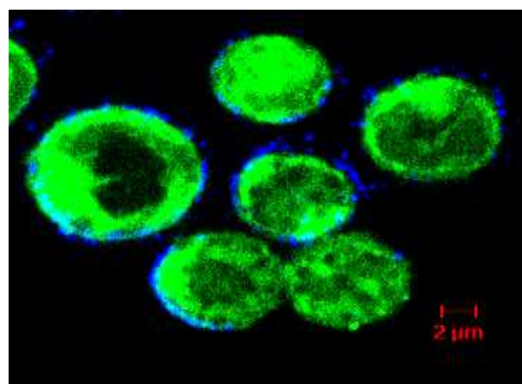


Bershteyn et al. *Soft Matter* (2008); Moon et al. *Nat. Mater.* (2011)

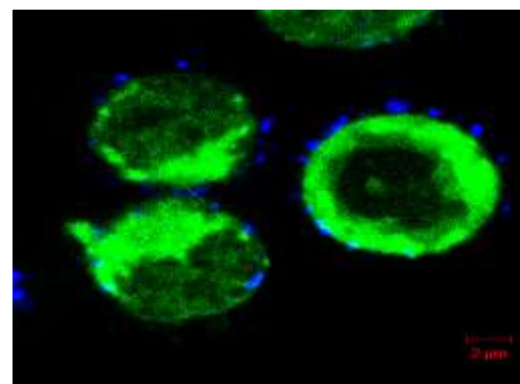
ICMVs



1200



600



150