SIV persistence in ART-treated infant rhesus macaques

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Infant monkeys ≠ Small adults

Monkeys ≠ Humans

However, the SIV/SHIV-rhesus macaque model can be used to enhance our understanding of virus persistence and, particularly, anatomic reservoirs that are difficult or impossible to study in humans.
“The major knowledge gaps for perinatal HIV infection are in understanding the mechanisms of latency in infants and children. The dynamics of HIV persistence in children are probably different than those in adults, owing to a number of factors, such as the type and numbers of target cells, the efficiency in clearing HIV-infected cells and pharmacokinetics of ART in blood and tissues…Given the difficulties in studying young children, development of infant animal models should be pursued.”
**Study design and aims**

- **Aim 1:** To demonstrate sustained suppression of viremia in SIV-infected, ART-treated infant RM

- **Aim 2:** To characterize cellular and anatomic SIV reservoirs in SIV-infected, ART-suppressed infant RM

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**2 doses of** $10^5$ **TCID$_{50}$ SIVmac251 (Oral gavage)**

**Four 4-month old rhesus macaques (RM)**

**ART**
Triple formulation daily injection (TFV, FTC, dolutegravir) for 6-9 months

**Blood samples collected throughout the study**

**Necropsy**

Week 5

Week 31-42
Plasma viral load

Undetectable plasma viral load (<60 copies/ml) after 4-22 weeks on ART maintained through the duration of the study
SIV DNA in PBMC

1-2 log decrease of SIV DNA levels in PBMC after ART

Significant contribution of the naïve and central memory CD4⁺ T-cells to the SIV reservoir
SIV DNA in lymphoid tissue
CD4⁺ T-cell subsets

Peripheral Blood
- NAIVE
- SCM
- CM
- EM

Spleen
- NAIVE
- SCM
- CM
- EM

Superficial LN
- NAIVE
- SCM
- CM
- TFH

Mesenteric LN
- NAIVE
- SCM
- CM
- TFH

retroperitoneal LN
- NAIVE
- SCM
- CM
- TFH

SLN
- 65%
- 17%
- 11%
- 7%

SIV DNA (copies/million CD4⁺ T-cells)

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SIV DNA in additional tissues

- PBMC
- Tonsil cells
- Thymus CD3+ CD4+CD8- cells
- Small intestine CD4+ T-cells
- Large intestine CD4+ T-cells

SIV DNA (copies/million cells)
Reduced levels of SIV RNA in tissues of ART-treated as compared to untreated viremic infant RM but similar levels in the brain and gut

Claire Deleage / Jake Estes
SIV DNA in the CNS (DNAscope)

RSi16 FC - DNAscope

A14115 Cerebrum - DNAscope
ARV levels in tissues

**LC-MS/MS**
- Front-end separation → Sensitive analysis of targeted compounds only
- Entire sample processed at once → Provides one value for the whole sample.

**IR-MALDESI: Infrared Matrix-Assisted Laser Desorption Electrospray Ionization**
- No front-end separation → *Simultaneous analysis of multiple compounds*
- Analyzes sample in small steps → *Distribution of compound within a sample can be measured*

**Mass Spectrometry Imaging Reveals Heterogeneous Efavirenz Distribution within Putative HIV Reservoirs**
Corbin G. Thompson,9 Mark T. Bokhart,5 Craig Sykes,3 Luciades Adamson,5 Yuri Fedorine,9 Paul A. Luciw,6 David C. Muddiman,9 Angela D. M. Kashuba,9 Eli P. Rosen*
Biodistribution of the ARVs in the lymph nodes

Ion maps show ARV overlay on Cholesterol

Heterogeneous distribution of both TFV and DTG with TFV concentrated in medullary sinuses
Biodistribution of the ARVs in the brain

No detection of ARVs in the brain by IR-MALDESI
Biodistribution of the ARVs in the colon

Heterogeneous distribution of DTG in the colon focusing near mucosa

LC-MS/MS ARV Concentrations in the Colon

File: 041916_CPAC 697_RH16 Colon.imdML
miz: 420.13660 ± 0.00105 Th ± 2.5 ppm
Normalization: none

ARV Concentration (ng/g tissue)

- TFV
- FTC
- DTG

ARV Concentrations in the Colon

RSi16 | RPi16 | RUh16 | RHi16
6.5e3 | 3e3 | 6e3

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Conclusions and perspectives

- ART was well tolerated and efficient with undetectable plasma viral load achieved within a similar time frame as HIV-infected infants.
- ART reduces SIV DNA levels in PBMC and RNA levels in most lymphoid tissues analyzed but not in the brain or gut.
- Heterogeneous drug penetration in lymph nodes and colon; very little drug present in the brain.
- Naïve and central memory CD4+ T cells appear to constitute a large proportion of the SIV DNA reservoir in ART-treated infant RM.

*This study establishes a model of pediatric SIV infection and viral suppression under ART in infant RM and provides an experimental in vivo platform to study reservoirs and test cure strategies in the period of infancy.*
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Low level of activation markers on naïve T-cells

Naïve: CD45RA+CCR7+CD95- (also CD28dim)
SCM: CD45RA+CCR7+CD95+
CM: CD28+CD95+CCR7+
EM: CD28+-CD95+CCR7-
Naïve CD4+ T cell infection

Brenchley et al J Virol 2004
(HIV-infected patients on and off ART)

Brenchley et al Blood 2012
(SIV-infected monkeys)
Naïve CD4+ T cell reservoir

SIV DNA in peripheral CD4\(^+\) T-cell subsets

Comparison to only previously published data on CD4\(^+\) T-cell subsets from perinatally HIV-infected patients

Luzuriaga, JID, 2014
ARV levels in fluids

Compared to NHP values

Compared to human values

- **DTG**
  - CPAC697 data vs NHP data
  - Dolutegravir 2.5mg/kg/day

- **FTC**
  - CPAC697 data vs NHP data
  - Emtricitabine 40mg/kg/day

- **PMPA**
  - CPAC697 data vs NHP data
  - PMPA 20mg/kg/day

- **Plasma**
- **CSF**
ARV levels in the brain

Very low levels of ARVs in the brain confirmed by LC-MS/MS
HIV-1 pediatric infection

<table>
<thead>
<tr>
<th>Global summary of the AIDS epidemic in children (&lt;15 years) – 2014</th>
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<tbody>
<tr>
<td>Number of children living with HIV in 2014</td>
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<tr>
<td>Children newly infected with HIV in 2014</td>
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<tr>
<td>AIDS death in children in 2014</td>
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- Antiretroviral therapy (ART) reduces mortality but is not curative

- HIV-1 persistence on ART
- Reservoir of long-lived latently-infected memory CD4⁺ T-cells
- Other cellular reservoirs (myeloid lineage)
- Anatomical drug sanctuaries?

Lifetime therapy needed
HIV-1 pediatric infection: can we cure?

Sustained HIV control in the absence of ART following 18 months of ART initiated 30h after birth in a perinatally HIV-infected child

- Opportunity to treat early following HIV infection
- Specific characteristics of the infant developing immune system
  - low abundance of memory CD4\(^+\) T-cells
  - more tolerogenic immune system

Need for a specific pediatric model to study HIV reservoirs