Frequent and ‘burst-like’ reactivation from latency in SIVmac239M infected macaques.

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Infection Analytics

Major questions.

• Frequency of reactivation from latency.
• How do we measure it?
• Early events in reactivation.
Measuring HIV reactivation from latency.

RESEARCH ARTICLE

HIV Reactivation from Latency after Treatment Interruption Occurs on Average Every 5-8 Days—Implications for HIV Remission

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Using time-to-detection after ATI

HIV reactivation from latency every 5-8 days (mean ≈ 6 days)

Using ‘reactivation founders’

\[ R = \frac{V_0 e^{gt_1}}{V_0 e^{gt_2}} = e^{g\Delta} \]

- Viral load
- Delay between founders
- Detection threshold

\[ g \]

\[ \Delta \]
Delay between reactivation events at least 3.6 days (2.1-7.5)

Frequency of reactivation

**Time-to-detection**
- Affected by drug washout
- One reading.
- Low statistical power.

**Reactivation founder ratio**
- Not affected by washout.
- Multiple readings.
- ? Statistical power.

Disadvantages of reactivation founders:

• Need to identify individual founders.
  (need SGA)

• Accurate ratios require lots of sequences.
  (not easy with SGA)
Using $\text{SIV}_{\text{mac}239}\text{M}$ to study Latency

Brandon Keele and team

AIDS and Cancer Virus Program, Leidos Biomedical Research Inc., Frederick National Laboratory, Frederick, MD.
Engineered SIVmac239M which Includes a 34bp Cassette for Variant Discrimination

SIVmac239

gag  pol  vif  tat  rev  nef

MluI

...TAAACGCCTATG...
...ATTTGCGCATAAC...

Primer dimer

CGCGGGCTACNNNNNNNNNNTGCAAG
CCGATGNNNNNNNNNAAACGTTGCGC

CGCGCTTGAANNNNNNNNNNGAAGCTT
GAACGTTNNNNNNNNNNNNNNNNNNNNNNN

(+)

...TAAACCGCGGCTACNNNNNNNNNNTGCAAG
...ATTTGCGCGCCGATGNNNNNNNNNNAAACGTT

(-)

...TAAACCGCGCTTGCAANNNNNNNNNNGGATGCC
...ATTTGCGCGAAGCTTNNNNNNNNNNCCATCGGC

Adding these 34nt provides $4^{10} (>1,000,000)$ potential

Phylogenetic Analysis Distinguishes Individual Variants
High dose infection (2.2 x 10^5 IU of SIVmac239M i.v.)

Early treatment (day 6, TFV/FTC/RAL for 80 days)
(Day 4 TFV/FTC/IND/RTV for 300-480 days)

Analysis of ‘reactivation founders’.
(Illumina sequencing of plasma virus)
- Pre-treatment plasma virus very diverse
  (size of clonotypes weakly correlated with stock, Spearman 0.07-0.18)
Rx d6, for 82 days
- 33-63 reactivation founders
Rx d4, for 300-480 days
• 3-6 reactivation founders
Rx d6, for 82 days
- 33-63 reactivation founders
- 10-27 reactivations per day.

Rx d4, for 300-480 days
- 3-6 reactivation founders
- 0.38-0.7 reactivations / day.

*ie: one every 1.4-2.6 days*
What predicts reactivation rate?

- Time of treatment? (viral load pre-treatment)
- Duration of treatment?
Peak viral load pre-ART predicts reactivation rate

Duration of ART?

A

Rate/day

100

10

1

0.1

$10^4$ $10^5$ $10^6$ $10^7$

Max VL

B

Rate

0.8

0.6

0.4

0.2

0.0

300 375 480

Day 4 Rx

C

Rate per $10^6$ cl/ml

10

8

6

4

2

0

82 300 375 480

All animals
Rate / VL
Detecting effects of interventions

Time-to-detection

- # patients with no rebound
  - time
Detecting effects of interventions

Time-to-detection

Ratio of founders

# patients with no rebound

0 10 20 30 40 50

0 2 4 6 8 10

time

0.0 0.2 0.4 0.6 0.8

Rx day 4

reactivation rate

LRA (schematic)
Detecting effects of interventions

- Time-to-detection
- Reactivation rate*

* Based on the six day 4 Rx
Using $\text{SIV}_{\text{mac}239\text{M}}$ to study dynamics of early reactivation events
Duration of viral production

Reactivation 27 times per day. (lasts ≈1 day)
Rapid ‘burst-like’ viral production

- 0.1 days
- 0.5 days
- 2 days
Understanding reactivation from latency

**SIV_{mac239M}**

![Graph showing reactivation rate](image1)

**Rx day 4**

![Graph showing number of patients](image2)

**Log ratio vs. Rebounder**

**Reduction in frequency of reactivation / size of reservoir (%)**

![Schematic diagram](image3)
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