Comprehensive longitudinal characterization of HIV-1 reservoir markers in patients on stable antiretroviral treatment

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Introduction

HIV infection is characterized by high levels of circulating viruses in the blood. Antiretroviral drugs are capable of suppressing HIV to undetectable levels. After stopping therapy, HIV rebounds. The viral reservoir is a key concept. Perelson et al - Nature. 1997; Finzi et al - Nature medicine. 1999; Archin et al - Curr Opin Infect Dis. 2014
HIV-1 reservoir

- Total HIV DNA
- Intact and integrated HIV DNA
- Replication competent reservoir
  - VOA

Bruner et al. Trends in microbiology. 2015; Ho et al Cell. 2013 0-51; Mexas et al-2012
Aims of the study

• To perform a comprehensive evaluation of viral reservoir markers quantified by digital PCR and VOA.

• To assess the long- and short-term dynamics and variations of HIV cellular markers by digital PCR.

• We used 25 patient samples with varying levels of HIV-1 burden

• Median 9 years (IQR: 6-12) ART
Patients' PBMCs

Quantification of HIV-1 reservoir with droplet digital PCR

RNA, DNA and 2LTR isolation

Baseline

Median 2.5 years

Time point 1

Patients' PBMCs

CD4 isolation

Viral outgrowth assay

Temporal changes

Assay variability

Time point 2

Patients' PBMCs

Alu PCR for integrated HIV-1 DNA

Quantification of HIV-1 reservoir with droplet digital PCR

Medan 30 days

Time point 2

Patients' PBMCs

RNA, DNA and 2LTR isolation

Quantification of HIV-1 reservoir with droplet digital PCR

Time point 3

Patients' PBMCs

RNA, DNA and 2LTR isolation

Quantification of HIV-1 reservoir with droplet digital PCR

Longitudinal dynamics of HIV-1 intracellular markers

**Total HIV-1 DNA**
- 2.5 years
- 30 days
- $p = 0.988$
- $p = 0.47$
- $p = 0.26$

**Unspliced HIV-1 RNA**
- 2.5 years
- 30 days
- $p = 0.063$
- $p = 0.28$
- $p = 0.32$

**2LTR circles**
- 2.5 years
- 30 days
- $p = 0.062$
- $p = 0.016$
- $p = 0.69$

**Multiply spliced HIV-1 RNA**
- 2.5 years
- 30 days
- $p = 0.64$
- $p = 0.11$
- $p = 0.34$
Viral outgrowth assay - VOA

Correlation

- Total HIV DNA log 10 cp/10^6 PBMCs
  - Spearman’s R² = 0.54
  - p = 0.019

- Integrated HIV DNA log 10 cp/10^6 PBMCs
  - Spearman’s R² = 0.44
  - p = 0.05
Integrated HIV-1 DNA

Correlation

$p = 0.001$
Spearman’s $R^2 = 0.28$

Correlation

$p = 0.002$
Spearman’s $R^2 = 0.85$
Bland-Altman analysis of HIV-1 quantification

Assessment of agreement in HIV-1 quantification between the PCR based methods and the viral outgrowth assay

Quantification bias (log10)
95% Limits of agreement
Bland-Altman analysis of HIV-1 quantification

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Quantification bias (log10)
95% Limits of agreement
Conclusion

• Our data confirms long-term stability of the size and dynamics of HIV-1 DNA levels

• The total pool of HIV-1 DNA correlated with VOA ➔ stable fraction of integrated HIV-1 is replication competent

• total HIV-1 DNA and VOA might predict each other ➔ further validation in treated patients

  » The agreement between PCR based and VOA is a function of the total HIV-1 reservoir size.

• A correlation was observed between the residual proviral mRNA transcription levels and integrated HIV-1 DNA
Acknowledgements

HTRU
Linos Vandekerckhove
Ward De Spiegelaere
Karen Vervisch
Eva Malatinkova
Pawel Bonczkowski
Wim Tryptoenstein
Marie-Angélique De Scheerder
Sofie Rutsaert
Clarissa Van Heck

UZGent virology department
Bruno Verhasselt

AIDS reference lab
Chris Verhofstede

The Ragon Institute of MGH, MIT and Harvard
Mathias Lichterfeld
Xu Yu
Maria Jose Buzon

To all patients participating in this study